Importance des traits clonaux dans la réponse à la défoliation et au pâturage chez des plantes herbacées.

Marie-Lise Benot

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Marie-Lise Benot

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devant le jury composé de :

Danièle MAGDA
DR INRA Castanet Tolosan / *rapporteur*

Marek SAMMUL
Dr Univ. Tartu, Estonie / *rapporteur*

Jan-Bernard BOUZILLÉ
Pr Univ. Rennes 1 / *examinateur*

Sandra LAVOREL
DR CNRS Grenoble / *examinateur*

Marie-Pascale PRUD'HOMME
Pr Univ. Caen / *examinateur*

Anne BONIS
CR CNRS Rennes / *directeur de thèse*

Cendrine MONY
MC Univ. Rennes 1/ *co-directeur de thèse*

Résumé

La clonalité chez les plantes correspond à la multiplication par voie végétative. Un individu clonal est constitué de l'ensemble de ses descendants, génétiquement identiques et potentiellement indépendants (ramets), généralement reliés entre eux par des connexions. L'intégration clonale confère aux plantes des propriétés particulières dont les principales sont la capacité à coloniser l'espace via différents types d'architectures, le stockage et le partage de ressources entre les ramets. Les plantes clonales dominent la matrice herbacée en prairies pâturées.

L'objectif de ce travail de thèse est de tester l'hypothèse selon laquelle le pâturage favorise les traits clonaux conférant aux plantes des capacités de résistance, notamment à la défoliation (*i.e.* pertes de tissus aériens) qu'il génère. Cette hypothèse a été testée au travers d'une approche pluridisciplinaire, combinant écologie des communautés, écophysiologie et modélisation.

Des relevés de terrain réalisés en prairies naturelles ont montré que le pâturage génère une défoliation homogène à l'échelle du fragment clonal (inférieure à un mètre) et agit comme un filtre sur les traits clonaux. L'étude couplée de la composition floristique et des traits clonaux, issus de base de données ou mesurés expérimentalement, suggère que le pâturage favorise les formes stolonifères et cespiteuses, tandis que les formes rhizomateuses dominent en conditions non pâturées. De plus, les coûts associés à la défoliation homogène limitent l'investissement dans la propagation clonale. La plasticité architecturale en réponse à la défoliation expérimentale s'avère néanmoins dépendante de contraintes structurales propres à l'espèce. Par conséquent, il n'y a pas de convergence vers un seul type d'architecture, mais il semble, au contraire, qu'une diversité d'architectures puisse s'exprimer en prairies pâturées. Enfin, bien que le pâturage défavorise les organes souterrains spécialisés dans le stockage (rhizomes), la constitution de réserves carbonées dans la base des tiges des ramets serait impliquée dans la résistance au pâturage.

D'après les résultats de simulations numériques, les formes clonales optimales en absence de défoliation et sous défoliation homogène sont similaires et tendent à produire des réseaux agrégés de ramets. Au contraire, des conditions de défoliation hétérogènes favoriseraient la dispersion spatiale des ramets.

De manière générale, le pâturage semble favoriser les formes clonales permettant de maximiser l'occupation de l'espace et la constitution de stocks de réserves rapidement mobilisables pour la repousse suite à la défoliation, tout en limitant l'investissement dans les structures clonales coûteuses.

Mots-clefs

Traits clonaux, architecture clonale, réserves carbonées, échelle d'hétérogénéité spatio-temporelle, stratégies de réponse, prairies naturelles, modèle individu-centré (IBM).

Abstract

Clonality in plant corresponds to vegetative multiplication. A clonal individual is composed of genetically identical and potentially autonomous descendents (ramets), generally linked altogether by connective stems. Clonal integration confers on plants singular properties, the main ones being the ability to colonize space through diverse clonal architectures, resource storage and resource sharing between ramets. Clonal plants are dominant in the vegetation of grazed meadows.

The objective of this thesis is to test the hypothesis that grazing should promote clonal traits conferring on plants resistance capacities, notably to grazing-induced defoliation (*i.e.* losses of above-ground tissues). This hypothesis was tested through a pluri-disciplinary approach, combining community ecology, ecophysiology and modelling.

In situ vegetation sampling carried out in natural prairies, demonstrated that grazing-induced defoliation is homogeneous at the scale of the clonal fragment (less than one meter) and acts as a filter on clonal traits. The combined investigation of specific composition and clonal traits, documented from databases or monitored experimentally, suggests that grazing promotes stoloniferous and caespitose growth forms, while rhizomatous growth forms dominate under ungrazed conditions. Moreover, costs associated to homogeneous defoliation decrease the investment in clonal propagation. However, architectural plasticity in response to experimental defoliation depends on species-specific structural constraints. Consequently, no convergence towards a single architecture was observed. On the contrary, a diversity of clonal architecture is likely to express in grazed meadows. Furthermore, although grazing disfavours belowground storage organs (rhizomes), carbon reserve making in the basis of ramet stems seems involved in grazing resistance.

According to the results of numerical simulations, optimal clonal growth forms in the absence of defoliation and under homogeneous defoliation are similar. They tend to produce aggregated ramet networks. By contrast, heterogeneous defoliation is likely to promote the spatial dispersion of ramets.

Grazing appears to favour clonal growth forms that enable to maximize spatial occupation and storage of resources, which can be mobilized readily after defoliation, while limiting the investment in costly clonal structures.

Key words

Clonal traits, clonal architecture, carbon reserves, scale of spatio-temporal heterogeneity, response strategies, natural meadows, individual-based model (IBM).

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Sommaire

"Plants using similar modes of clonal growth can be found in different habitats, but also plants with different modes of clonal growth can be found together in the same habitat. The pertinent ecological question is whether the observed distribution of trait – environment relationship reflects an ecological adaptation to individual habitats that can help us interpret the evolution of clonality."

Klimeš *et al*. 1997

INTRODUCTION GÉNÉRALE

1. La clonalité chez les plantes

1.1. Présentation de la clonalité

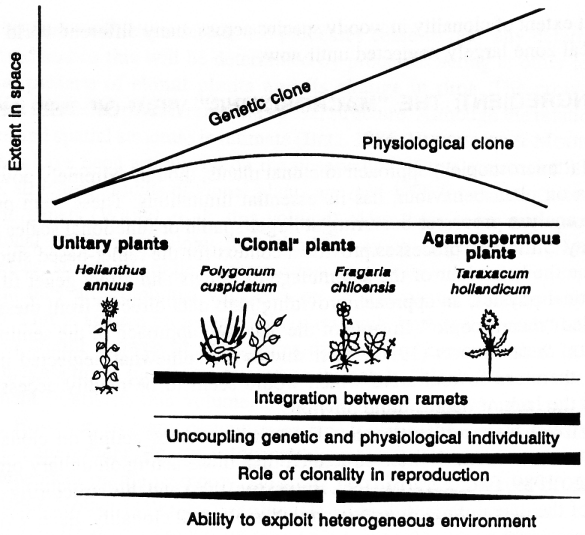
1.1.1. Définition

La **clonalité** est très répandue chez les plantes, en particulier chez les plantes vasculaires. Ainsi, environ 70% des espèces végétales des climats tempérés ont été recensées comme clonales (van Groenendel & de Kroon 1990, Klimeš *et al.* 1997). Elle peut être définie comme « la capacité à se propager et à produire des descendants par voie végétative » (Bell 1984). Elle est synonyme de **croissance clonale** ou encore de **multiplication végétative** (Schmid 1990). La clonalité ainsi définie se distingue de la **reproduction clonale** (**apomixie** au sens large ou **agamospermie**, généralement plus employée chez les plantes). Cette dernière correspond à la production de descendants génétiquement identiques à partir d'une seule cellule somatique. Dans ce cas, la méiose et la fécondation n'ont pas lieu, et sont remplacées par une simple mitose. Clonalité et agamospermie présentent des différences biologiques et écologiques (Herben *et al.* 1994 ; encadré 1). Le présent manuscrit ne portera que sur la croissance clonale.

La croissance clonale repose principalement sur l'organisation modulaire, très développée chez les plantes. Un **individu modulaire** est constitué de la répétition potentiellement infinie de sous-unités multi-cellulaires et génétiquement identiques, les **modules** (Harper 1977). Ces derniers présentent une certaine autonomie (Tuomi & Vuorisalo 1989). Ceci implique notamment qu'ils ont la capacité d'acquérir au moins une partie des ressources qui sont nécessaires à leur fonctionnement et de répondre aux conditions environnementales locales, séparément du reste de l'individu (Tuomi & Vuorisalo 1989).

Cependant, bien que les modules présentent une semi-autonomie, ils ne pourraient pas se développer indépendamment du reste de la plante (*e.g.* branches des arbres). La clonalité repose sur une modularité de second ordre (Alpert & Stuefer 1997). Les modules clonaux (ou **ramets**, Harper 1977) sont eux-mêmes constitués de plusieurs modules. Ils sont potentiellement indépendants, car ils possèdent leurs propres systèmes racinaire et aérien. Ainsi, les ramets peuvent survivre et se développer même après séparation de l'individu. L'individu clonal (individu génétique ou **genet**) consiste en l'ensemble des structures (ramets et autres structures clonales que nous évoquerons ultérieurement) dérivées d'un zygote unique (Harper & White 1974).

Encadré 1. Deux visions de la clonalité : le concept de clone génétique et de clone physiologique proposé par Herben *et al.* (1994).



La figure ci-dessus, proposée par Herben *et al.* (1994) illustre la notion d'individu clonal et de fragment clonal (ensemble de ramets interconnectés entre eux par intégration clonale).

Chez les plantes unitaires, l'expansion de l'individu dans l'espace (*i.e.* dans le plan horizontal) est très faible.

Chez les plantes clonales, au sens où nous l'entendrons dans le présent manuscrit (à savoir des plantes qui ont la capacité de se multiplier par voie végétative), le degré d'intégration entre les ramets est variable. On peut faire la distinction entre le clone « physiologique » (*i.e.* un fragment clonal de ramets reliés entre eux par intégration clonale) et le clone « génétique » (*i.e.* genet issu d'une graine et correspondant à l'ensemble des fragments clonaux produits par multiplication végétative et génétiquement identiques). Les deux cas représentés ici diffèrent selon leur degré d'intégration clonale, à savoir la durée de vie et de fonctionnalité des connexions (cf. stratégies clonales). La clonalité permet une expansion spatiale des fragments clonaux et des genets maximale dans le cas de connexions dont la capacité d'élongation et la durée de vie sont longues. Ces formes clonales sont également celles qui permettent une exploitation de l'hétérogénéité spatiale efficace. Leur expansion spatiale est moindre dans les cas où les connexions sont courtes (ce qui correspond le plus souvent à un fort degré d'intégration clonale) ou dans les cas où leur durée de vie est courte (fragmentation importante générant des fragments clonaux de petite taille). A contrario, dans ce dernier cas, l'expansion de l'individu génétique est importante. Chez les plantes clonales, la multiplication végétative n'est pas le seul moyen de reproduction, la plupart des espèces se reproduisant également par voie sexuée.

Dans le cas d'agamospermie (ou apomixie), l'individu produit des graines qui lui sont génétiquement identiques (méiose et fécondation remplacées par la mitose). L'expansion spatiale du clone est donc maximale (dispersion par les graines). A l'inverse, l'intégration clonale entre les ramets est quasi nulle. Chez ces plantes, l'agamospermie[1] est le mode de reproduction principal.

[1] Notons qu'Herben *et al*. (1994) utilisent dans cette figure le terme de clonalité à la fois pour désigner la multiplication végétative et l'agamospermie. Dans le présent manuscrit, nous distinguerons ces deux notions, en réservant le terme de clonalité à la multiplication végétative.

1.1.2. Origine phylogénétique

L'origine phylogénétique de la clonalité demeure encore aujourd'hui mal connue. La capacité de croissance clonale chez les plantes serait un caractère ancestral, puisque présent chez les *Rhyniopsida*, phylum à l'origine des autres groupes de plantes vasculaires (Mogie & Hutchings 1990). Pourtant, et bien qu'à l'heure actuelle les angiospermes clonaux soient relativement communs, ceux-ci dériveraient d'un ancêtre non clonal (Fischer & van Kleunen 2002). Un consensus existe selon lequel la clonalité chez les angiospermes aurait une origine multiple et serait apparue indépendamment dans diverses lignées (Mogie & Hutchings 1990, Eriksson 1992, Klimeš *et al.* 1997). Cependant, tandis que la présence de la clonalité chez les dicotylédones primitives reste controversée, elle serait un caractère présent dès l'origine de l'apparition des monocotylédones. Par conséquent, la capacité de croissance clonale est aujourd'hui plus communément répandue chez les monocotylédones que chez les dicotylédones.

1.2. L'individu clonal

1.2.1. Une notion complexe

Pourtant claire en théorie, la notion d'individu est, en pratique, rendue difficile lorsque l'on s'intéresse aux plantes clonales. L'individu clonal, possédant un génotype unique et soumis à sélection, est l'ensemble des structures issues d'une seule graine. Du fait de leur potentielle autonomie, des ramets séparés du reste du genet, peuvent à leur tour se multiplier par voie végétative. Bien que physiquement déconnectés, les divers **fragments clonaux** (Eriksson & Jerling 1990) ainsi produits appartiennent à un seul et unique individu clonal (Vuorisalo & Tuomi 1986). Les points de vue génétique et écologique de l'individu clonal peuvent varier (encadré 1). Ainsi, de nombreuses études expérimentales s'intéressant à la clonalité considèrent comme genets des ensembles de ramets clonaux interconnectés. Cette appellation est erronée puisqu'il s'agit en réalité de plusieurs fragments appartenant parfois à un seul individu clonal (Herben *et al.* 1994). En outre, excepté dans les cas où les ramets restent physiquement reliés entre eux, l'identification d'un individu clonal *in situ* est difficile et nécessite le recours à des outils génétiques. La prise en compte et la description de la clonalité *in situ* sont souvent contraintes à se limiter à l'étude des caractéristiques des ramets, considérés alors comme l'unité fonctionnelle d'intérêt principal (Weiher *et al.* 1999) et, éventuellement à celles des structures clonales qui leur sont directement rattachées.

1.2.2. Organes clonaux et intégration physique

La croissance clonale peut s'exprimer de manière variée, incluant la fragmentation de tiges ou de racines, la production de bourgeons adventifs ou d'organes spécialisés (tiges modifiées, bulbes, tubercules ; Klimeš *et al.* 1997). Parmi ces derniers, les organes d'origine caulinaire sont les plus fréquemment répandus (van Groenendael *et al.* 1996; Klimeš *et al.* 1997; Klimešovà & Klimeš 2008). Il s'agit notamment de tiges modifiées, à développement plagiotrope (horizontal), souvent désignées sous le terme général de **connexions**. Ces tiges peuvent être souterraines (**rhizomes**) ou aériennes (**stolons** et tiges rampantes ou **runners**[2]).

La production de connexions constitue l'**intégration physique** (encadré 1). Elle est à l'origine de formes de croissance clonale en réseaux de ramets reliés entre eux par des portions de connexions, les '***spacers***'[3] (Bell 1984 ; Fig. I). Ces formes clonales font l'objet d'études nombreuses (Klimeš *et al.* 1997), car elles confèrent aux plantes des propriétés physiologiques et écologiques particulières (cf partie « propriétés clonales »).

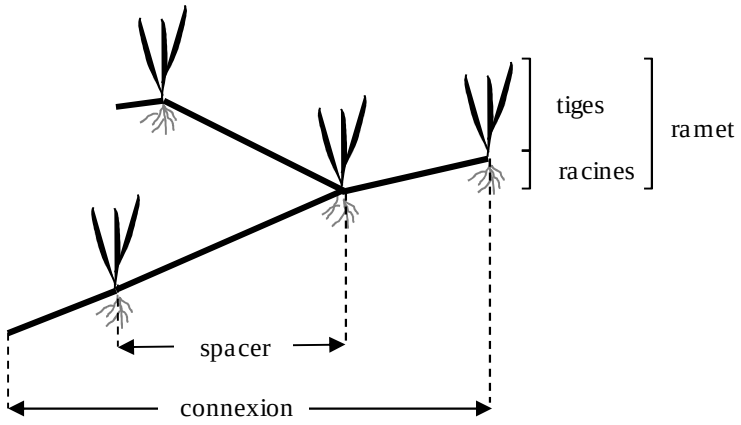


Fig. I. Forme de croissance clonale en réseau. Les ramets sont reliés entre eux par des portions de connexions, les '*spacers*' (intégration clonale).

1.2.3. Expansion dans le temps et dans l'espace

La clonalité confère aux plantes la possibilité de s'étendre dans le temps et dans l'espace. Ainsi, bien que les ramets aient une durée de vie limitée, la clonalité peut permettre à l'individu clonal d'échapper à la sénescence, et lui conférer une durée de vie et une expansion latérale potentiellement illimitées, à condition qu'il soit constitué, à chaque instant, d'un ramet, ou au moins d'un bourgeon, viable et capable de se multiplier par voie végétative

[2] Tiges orthotropes devenant plagiotropes au cours de leur développement, et produisant des ramets au niveau des nœuds

[3] Bien qu'il soit parfois employé comme synonyme de connexions, le terme anglophone '*spacer*' désigne généralement la fraction de connexion reliant deux ramets consécutifs. C'est cette définition qui sera préférée dans le présent manuscrit, afin de le distinguer des connexions (*i.e.* ensemble de spacers).

(Sackville Hamilton *et al.* 1987, Wikberg 1995). Ainsi, des fragments clonaux de fougère aigle (*Ptertidium aquilinum*) d'envergure supérieure à 500 m (Harper 1977, p516), voire même allant jusqu'à 1km et datés de 1000 ans (McLellan *et al.* 1997) ont été recensés. L'âge d'un clone de *Carex curvula* aurait été estimé à plus de 2000 ans (McLellan *et al.* 1997), celui d'un clone de peuplier (*Populus tremuloides*), à 10000 ans (Jónsdóttir *et al.* 2000). L'exemple le plus marquant reste à l'heure actuelle celui d'un clone de peuplier (*Populus tremuloides*) daté à un million d'années et s'étendant sur une surface de 43 ha (McLellan *et al.* 1997) !

De manière plus générale, la clonalité est donc souvent associée au mode de vie pérenne. Cependant, elle peut également s'exprimer chez des espèces annuelles (ramets et organes clonaux annuels). Dans ce cas, l'ensemble du genet meurt à la fin de la saison de croissance. Il s'agit néanmoins d'un individu clonal, celui-ci ayant la capacité de produire des descendants potentiellement autonomes par voie végétative. Cependant, la transmission de ses gènes aux générations futures repose exclusivement sur la reproduction sexuée des ramets. La clonalité joue, dans ce cas, un rôle dans la fitness de l'individu, en multipliant le nombre d'unités pouvant se reproduire sexuellement (les ramets).

1.3. La fitness chez les plantes clonales

1.3.1. Clonalité et reproduction sexuée

Bien qu'ayant la capacité de se reproduire par voie végétative, les plantes clonales se reproduisent également, dans la plupart des cas, par voie sexuée (reproduction mixte). Ainsi que le suggère l'existence de variations génétiques au sein des populations de plantes clonales, la production de graines, permettant le brassage génétique, reste un trait important dans l'histoire de vie des plantes clonales (Eriksson 1989, 1997). La reproduction sexuée est indispensable à l'établissement d'un nouveau genet. L'implication de la reproduction sexuée dans la dynamique des populations de plantes clonales varie selon un gradient aux extrémités duquel ont été proposées deux stratégies (Eriksson 1989). La stratégie **ISR** (**Initial Seed Recruitment**) ne fait intervenir la reproduction sexuée qu'une seule fois, lors de l'établissement de la population qui croît, par la suite, exclusivement par multiplication clonale. Dans ce cas, la population est composée d'un nombre fini de genets, qui tend à diminuer avec au fur et à mesure que l'âge de la population augmente (McLellan *et al.* 1997). A l'autre extrême, la stratégie **RSR (Repeated Seed Recruitment**) repose sur le recrutement de nouveaux individus par voie sexuée de manière répétée au sein d'une population établie.

Le degré d'investissement dans la reproduction sexuée et la multiplication clonale est très variable selon les espèces (Eriksson 1997). L'investissement dans l'un ou l'autre de ces modes de reproduction, ainsi que leur valeur adaptative sont depuis longtemps étudiés, et demeurent, à l'heure actuelle un sujet phare de la recherche sur les plantes clonales. La question majeure est de déterminer les forces qui favorisent leur expression respective. Un consensus théorique tend à admettre l'existence de compromis entre reproduction sexuée et croissance clonale, et a été confirmé par de nombreuses données empiriques (Eriksson 1997, Piquot *et al.* 1998, Prati & Schmid 2000, Fischer & van Kleunen 2002, Stöcklin & Winkler 2004), bien que certaines études aient abouti aux conclusions inverses (*e.g.* Cain & Damman 1997). Outre son implication dans la réparation des dommages de l'ADN, deux avantages de la reproduction sexuée permettraient son maintien chez les plantes clonales (Eriksson 1997) : (1) l'efficacité de dispersion spatiale par les graines et (2) les bénéfices, à court terme, de la variation génétique au sein d'une population. Celle-ci permet notamment de faire face à d'éventuels changements environnementaux imprévisibles, mais également de limiter la compétition intra-individuelle (Zobel 2008). Les conditions environnementales, notamment l'hétérogénéité spatio-temporelle et, plus particulièrement son échelle, seraient des éléments majeurs influençant l'équilibre entre les deux modes de reproduction. Dans les paysages hétérogènes (hétérogénéité grossière), la dispersion par les graines sur de grandes distances serait nécessaire à la colonisation de nouveaux habitats et au maintien de la structure des métapopulations. La dispersion par voie clonale, quant à elle permettrait la colonisation rapide de micro-habitats (hétérogénéité fine), notamment après perturbation (Fahrig *et al.* 1994) et le maintien des populations (Piquot *et al.* 1998, Winkler & Fischer 2002, Stöcklin & Winkler 2004).

1.3.2. Différentes approches de la fitness

La fitness est classiquement considérée comme la contribution d'un individu aux futures générations. Cette notion est cependant relativement vague et peut avoir plusieurs significations, rendant son estimation difficile. Chez les plantes clonales, la mesure de la fitness est rendue plus complexe encore, principalement du fait de leur organisation hiérarchique (du ramet au genet) et de leur capacité à produire des descendants par voie végétative et sexuée (Tuomi & Vuorisalo 1989).

Une première étape est de déterminer l'échelle à laquelle doit être estimée la fitness (Tuomi & Vuorisalo 1989, Wikberg 1995) En théorie, l'unité à considérer est l'individu clonal (le genet). Cependant, un genet peut être constitué de plusieurs ramets ou fragments

clonaux, plus ou moins dispersés dans l'espace et, par conséquent, situés dans des environnements différents. Ceci est d'autant plus marqué dans des environnements hétérogènes. La fitness d'un ramet peut différer de celle d'un autre ramet situé dans un micro-habitat différent (Winkler & Fischer 1999). L'évaluation du nombre de ramets et de leur propre fitness semble donc permettre une approximation relativement fiable de la fitness du genet. Cependant, cette estimation ne prend pas en compte la limitation des ressources provoquée par l'augmentation de la densité des ramets au cours du développement de l'individu clonal. En effet, la compétition inter-ramets peut limiter leur croissance ou le contrôle de leur production par le fragment clonal (de Kroon & Kwant 1991, Winkler & Fischer 1999). Plus que le nombre de ramets produits, leur densité, prenant en compte la surface occupée par le genet, apparaît un élément important de l'estimation de la fitness d'une plante clonale.

Une seconde difficulté pour évaluer la fitness d'un individu clonal repose sur la capacité de reproduction mixte des plantes clonales. Excepté dans le cas de mutations, les ramets sont génétiquement identiques à l'individu parent, tandis que les graines, issues d'un brassage génétique, ne partagent que la moitié des allèles d'un genet. En outre, les allocations à la croissance clonale et à la reproduction sexuée peuvent être contraintes par des compromis, variables selon les espèces ou les conditions environnementales.

La mesure de la fitness d'un individu clonal est donc extrêmement complexe, voire impossible. Plutôt que de fitness, nous parlerons donc de **performance** et, plus particulièrement de **performance clonale**, dans les cas où la participation de la reproduction sexuée dans la production de descendants est omise. La biomasse ou le taux d'accroissement relatif (RGR, Relative Growth Rate), le nombre de ramets, le taux de multiplication clonale (prenant en compte à la fois le nombre de ramets et des estimateurs de leur propre performance) ainsi que la surface couverte par le genet à un instant donné sont les estimateurs les plus couramment utilisés (Liao *et al.* 2003, Santamaria *et al.* 2003, Puijalon *et al.* 2005, Monro *et al.* 2007).

1.4. Propriétés clonales

1.4.1. Intégration clonale

Chez les formes clonales en réseaux, les ramets sont connectés entre eux par les connexions (**intégration physique**). Lorsqu'elles sont pourvues de vaisseaux fonctionnels, ces connexions peuvent permettre la translocation de nutriments et d'eau (sève brute) ou

d'assimilats (sève élaborée) (Oborny *et al.* 2001). Ce partage des ressources correspond à l'**intégration physiologique**. Mais les connexions peuvent également être le siège du transport de substances non nutritives telles que des phytohormones permettant le contrôle de la croissance au sein du clone (Kun & Oborny 2003), des substances messagers, notamment impliquées dans l'induction des réponses à des signaux externes (Stuefer *et al.* 2004, Gómez & Stuefer 2006, Gómez *et al.* 2007), mais également de maladies ou de pathogènes (Wennström & Ericson 1992, Stuefer *et al.* 2004, Koubek & Herben 2008). L'intégration physique, notamment les connexions aériennes (stolons, tiges rampantes), pourrait même faciliter la mobilité des micro-herbivores (*e.g.* invertébrés), en jouant le rôle de ponts entre les ramets (Stuefer *et al.* 2004).

L'intégration physiologique est dépendante de l'anatomie vasculaire, à savoir les liens existant entre les vaisseaux (Price *et al.* 1992). Ainsi, le transport de substances peut n'avoir lieu qu'au sein d'unités d'intégration (**IPU**, **Integrated Physiological Units**, Watson & Casper 1984), conduisant à la sectorialité de l'intégration.

En général, les flux de xylème et de phloème sont orientés par les relations sources-puits internes à la plante (Marshall 1990). Ainsi, les transports de sève sont généralement orientés vers les zones de croissance active (Kelly 1995), à savoir les ramets non enracinés ou immatures (Marshall 1990, Price *et al.* 1992, Alpert 1996) ou les extrémités des connexions (transport acropétal, Landa *et al.* 1992, Price & Hutchings 1992a, D'Hertefeldt & Jónsdóttir 1999). Cependant, le transport de produits de la photosynthèse peut également avoir lieu, dans une moindre mesure, en direction des ramets ou des connexions matures (transport basipétal, Jónsdóttir & Callaghan 1989, Price *et al.* 1992, D'Hertefeldt & Jónsdóttir 1999). Des conditions externes à la plante peuvent altérer ces relations sources-puits et augmenter le degré d'intégration entre les IPUs (Price *et al.* 1992). Ainsi l'intégration physique permet le soutien des ramets stressés ou endommagés (*e.g.* ressources limitantes, Hartnett & Bazzaz 1983, défoliation, Jónsdóttir & Callaghan 1989). Cependant, les ramets juvéniles restent en général des puits plus forts que les ramets plus âgés (Jónsdóttir & Callaghan 1989, Alpert 1996). Ce soutien est limité dans le temps et s'arrête rapidement après plusieurs évènements de défoliation (Jónsdóttir & Callaghan 1989). L'influence des facteurs externes sur les caractéristiques de l'intégration physiologique (direction des flux, distance d'intégration, etc.) reste donc limitée par les contraintes internes à la plante (Price & Hutchings 1992a, b).

1.4.2. Architecture clonale

Dans le cas des formes de croissance clonale intégratrices ou en réseau, l'architecture clonale (*i.e.* la structure et la forme du réseau de connexions) détermine la distribution spatiale des ramets, et ainsi l'acquisition des ressources (Huber *et al.* 1999). Le réseau clonal est hiérarchisé et les connexions distinguées selon leur ordre. Les connexions formées à partir du ramet initial sont dites primaires. Les connexions d'ordres supérieurs (secondaires, tertiaires, etc.) sont généralement regroupées sous le terme de ramifications[4]. L'architecture d'un individu clonal est déterminée par des règles de croissance, telles que les patrons d'élongation *vs*. ramification (taux d'élongation, taux de ramification, angle de ramification) et la distance inter-ramets, *i.e.* la longueur de connexion reliant deux ramets consécutifs[5] (Bell & Tomlinson 1980, Oborny & Cain 1997, Marbà & Duarte 1998, Sintes *et al.* 2005, 2007, Brun *et al.* 2007).

1.4.3. Plasticité intra-individuelle et réponse à l'hétérogénéité environnementale

La plasticité phénotypique correspond à l'aptitude d'un génotype d'exprimer des phénotypes variables en fonction des conditions environnementales (Bradshaw 1965). De part leur organisation modulaire, les plantes exprimeraient la plasticité phénotypique non pas à l'échelle individuelle, mais à l'échelle intra-individuelle (ou sub-individuelle, de Kroon *et al.* 2005). Chaque module aurait la capacité de répondre de manière locale et semi-autonome aux micro-conditions environnementales dans lesquelles il se trouve. La plasticité individuelle résulterait de l'intégration des réponses plastiques modulaires (de Kroon *et al.* 2005). Cette plasticité intra-individuelle a été plusieurs fois démontrée chez des plantes non-clonales, tant au niveau racinaire, en réponse à la micro-hétérogénéité des nutriments (Birch & Hutchings 1994, Hutchings & de Kroon 1994) qu'au niveau des tiges et des feuilles, en réponse notamment aux conditions lumineuses locales (Huber & Hutchings 1997, Hutchings & de Kroon 1994). A l'échelle individuelle, ces réponses localisées peuvent être modulées par le degré d'intégration physiologique entre les modules (Sachs & Novoplansky 1997).

La plasticité intra-individuelle serait donc particulièrement avantageuse chez les plantes clonales, puisqu'elle leur offrirait la capacité d'exploiter efficacement des milieux

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[4] En anglais, le terme '***branching***' est généralement employé pour désigner le processus de ramification. Les ramifications sont donc dénommées '***branches***' (*e.g.* '***primary connections***' *vs.* '***branch connections***').

[5] 'Distance inter-ramet ('*inter-ramet distance*') et '*spacer length*' sont synonymes. A ne pas confondre avec la longueur des entre-nœuds ('*internode lenght*') 'spacer' pouvant être constitué de plusieurs entre-nœuds.

hétérogènes (Hutchings 1999). La plasticité intra-individuelle, chez les plantes clonales, peut s'exprimer tant au niveau du réseau de connexions et de l'architecture clonale (**foraging**) qu'à l'échelle du ramet (**spécialisation**) (Hutchings & de Kroon 1994, de Kroon & Hutchings 1995, McLellan *et al.* 1997)

1.4.3.1. Capacité de foraging

Le foraging peut être défini comme « le processus par lequel un organisme explore son habitat, lui permettant d'en augmenter l'exploitation et l'acquisition des ressources » (définition adaptée de de Kroon & Hutchings 1995). Chez les plantes, cette propriété correspond à la capacité de positionner préférentiellement les structures permettant l'acquisition des ressources (*e.g.* racines, organes photosynthétiques) là où celles-ci sont abondantes (foraging au sens large, Hutchings & de Kroon 1994). Chez les plantes clonales, le foraging est souvent considéré comme le positionnement des ramets dans les sites favorables, reposant sur la plasticité architecturale. Ainsi, un fort degré de ramification et des entre-nœuds courts permettraient l'installation d'un grand nombre de ramets dans les zones favorables, *i.e.* riches en ressources. A l'inverse, des formes clonales avec des connexions peu ramifiées et des entre-nœuds longs constitueraient une stratégie d'évitement des zones défavorables, *i.e.* pauvres en ressources (Slade & Hutchings 1987a, b, López *et al.* 1994 ; Fig. I).

Cette plasticité des connexions a été démontrée au sein d'un même fragment clonal en environnement hétérogène (Hutchings & de Kroon 1994), notamment en réponse à la disponibilité en nutriments (Wijesinghe & Hutchings 1999, Roiloa & Retuerto 2006) ou à la compétition (Eriksson 1986, MacDonald & Lieffers 1993, Kleijn & van Groenendael 1999, van Kleunen & Fischer 2001, Macek & Lepš 2003). Bien que les mécanismes de foraging soient peu connus, plusieurs auteurs ont suggéré que les apex des connexions en croissance auraient la capacité de percevoir la qualité de l'environnement (Wijesinghe & Hutchings 1999, Eriksson 1986). Selon la qualité perçue, soit l'entre-nœud poursuivrait son élongation (en conditions de mauvaise qualité) soit un nœud et un ramet seraient produits (en conditions de bonne qualité).

La réponse architecturale en conditions hétérogène apparaît cependant limitée, notamment par des contraintes structurales et des variations endogènes, indépendantes de l'environnement (de Kroon & Knops 1990), le foraging n'ayant été démontré que chez un nombre limité d'espèces.

1.4.3.2. Spécialisation des ramets et division du travail

La plasticité morphologique et physiologique des ramets en réponse à la disponibilité locale en ressources a été largement démontrée, et suggérée comme un élément complémentaire, voire prédominant sur la capacité de foraging (de Kroon & Hutchings 1995). Chez des plantes non-clonales, ou chez des individus clonaux se développant en conditions homogènes, il est généralement attendu que les ramets maximisent la capture des ressources rares et limitantes pour la croissance de la plante. Ainsi, en conditions ombragées, les ramets investissent dans la production de feuilles larges, ou dans l'élongation des pétioles afin de capter un maximum de lumière. Inversement, si ce sont les ressources édaphiques qui sont limitantes, les ramets présentent un appareil racinaire très développé. Ce phénomène correspond à la spécialisation pour les ressources rares (*specialization for scarcity*, Alpert & Stuefer 1997 ; Fig. II). En conditions hétérogènes, tous les ramets d'un même clone n'ont pas accès à la même quantité de ressources. Il a été démontré que les ramets situés dans les micro-sites riches tendaient à se spécialiser dans l'acquisition des ressources abondantes (*specialization for abundance*, Alpert & Stuefer 1997 ; Fig. II). Les ressources disponibles en grande quantité peuvent ainsi être redistribuées à l'ensemble du clone, notamment aux ramets qui en manquent. Cependant, cette propriété s'exprime principalement dans le cas où deux ressources sont distribuées de manière hétérogène et inversée (hétérogénéité complémentaire des ressources, Stuefer 1996, Alpert & Stuefer 1997). Par exemple, des ramets situés dans des sites ombragés mais riches en nutriments investissent préférentiellement dans la production de racines, tandis que les ramets qui leur sont connectés, mais se développant en milieu non ombragé et pauvre en nutriments, investissent dans la production de tissus photosynthétiques. Cette « **division du travail** » permet une acquisition importante des ressources et une exploitation maximale des conditions hétérogènes. La spécialisation peut également avoir lieu d'un point de vue physiologique. Ainsi, il a été suggéré que l'augmentation de la capacité photosynthétique ou du taux d'acquisition des nutriments permettaient aux ramets « sources » de répondre à la demande des ramets « puits » (Roiloa & Retuerto, 2006). Du fait de leur mise en place rapide, les réponses physiologiques seraient plus efficaces que les réponses morphologiques face à des changements ponctuels et imprévisibles des conditions environnementales tels que les pulses de nutriments (Hutchings & de Kroon 1994). Qu'il soit morphologique ou physiologique, ce type de spécialisation fonctionnelle des ramets est dépendant des conditions environnementales, et est habituellement désigné comme **spécialisation « induite par les conditions environnementales** », « **plastique** » ou « **spatiale** » (Stuefer 1998 ; Fig. II).

La division du travail peut également s'exprimer entre ramets de stade ontégénétique différent. Elle est, dans ce cas, fonction des capacités propres aux ramets mais indépendante des conditions environnementales. C'est la **spécialisation** « **développementale** » ou « **programmée** ». Chez *Carex bigelowii* (Cyperaceae), Jónsdóttir & Callaghan (1988, 1990) ont montré que les ramets âgés, dépourvus de système aérien étaient spécialisés dans l'acquisition des ressources édaphiques grâce à un réseau racinaire très développé. Inversement, les ramets plus jeunes aux racines peu développées, fournissent les produits de photosynthèse aux autres parties du clone. L'allocation des substances de défense contre les herbivores peut elle aussi être modulée par le stade de développement des ramets. Ainsi, Bråthen *et al.* (2004) ont démontré, au sein de fragments clonaux de *Carex stans*, que la quantité ainsi que la toxicité des composés de défense étaient plus importantes dans les ramets fleuris que dans les ramets végétatifs. Cette allocation serait avantageuse, car elle permettrait de protéger du pâturage par les lemmings, les fleurs et les graines, dont la production est coûteuse en énergie.

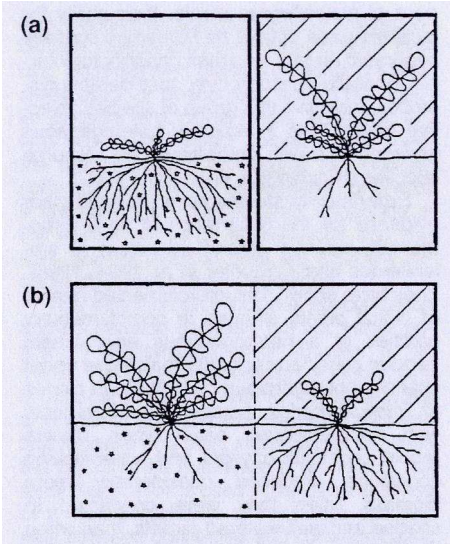

Fig. II. Division du travail induite par les conditions environnementales. (a) Spécialisation fonctionnelle pour les ressources localement rares chez les plantes non clonales ou des ramets isolés d'une plante clonale. (b) Spécialisation fonctionnelle pour les ressources localement abondantes chez un fragment clonal. Les hachures (partie aérienne) indiquent une limitation de la lumière, les étoiles (partie souterraine) indiquent une limitation en eau. D'après Stuefer (1998).

La séparation de l'allocation à la reproduction sexuée et à la multiplication clonale entre les ramets d'un même fragment clonal (spécialisation dans le mode de reproduction), bien que suggérée par les compromis existant entre ces deux fonctions, a rarement été démontrée. D'après une étude réalisée par Charpentier & Stuefer (1999), cette division du travail entre ramets de stades ontogénétiques différents s'exprimerait au cours d'une seule saison de croissance dans des fragments clonaux de *Scirpus maritimus*. Ainsi, les ramets les

plus âgés (situés à la base du rhizome) se spécialisent dans la reproduction sexuée, les ramets les plus jeunes (ramets terminaux dépourvus de feuilles), dans la fonction de stockage de réserves, les ramets végétatifs spécialisés dans la photosynthèse (ramets possédant un appareil aérien) se trouvant en position intermédiaire (Charpentier & Stuefer 1999). Cependant, la signification écologique de cette spécialisation développementale demeure inexpliquée et non systématique. Par exemple, pour des fragments clonaux cultivés en pots, la floraison des ramets pouvait se produire quelle que soit leur position sur le rhizome, indiquant une certaine plasticité de cette fonction (Charpentier & Stuefer 1999).

La division du travail nécessitant des flux de sève entre ramets ne peut être effective qu'au sein de fragments clonaux chez lesquels l'intégration physiologique et le partage des ressources entre ramets est efficace (cf. § intégration clonale).

1.4.4. Stockage de substances de réserves

La mise en place de réserves correspond au stockage de ressources carbonées et/ou azotées (Klimeš & Klimešová 2002), non directement assimilables par la plante mais remobilisables pour sa croissance ultérieure (Chapin *et al.* 1990). Tous les organes des plantes peuvent potentiellement accumuler des réserves, mais les stocks à long terme, chez les plantes non clonales, sont généralement établis dans les racines (van der Meijden *et al.* 1988).Chez les plantes clonales, les potentialités de mise en place de réserves sont accrues par la production d'organes spécialisés (*e.g.* stolons, rhizomes, bulbes, tubercules, etc.) et de ramets nombreux (Suzuki & Hutchings 1997). Parmi les plantes clonales, les capacités de stockage de réserves ne sont cependant pas équivalentes, certaines formes ou stratégies clonales étant, de ce point de vue, plus efficaces.

Les rhizomes, bulbes et tubercules sont généralement considérés comme les organes clonaux les plus impliqués dans le stockage de réserves (Hartnett 1989, Suzuki & Stuefer 1999, Asaeda *et al.* 2006). Ainsi, dans une étude sur *Cynodon dactylon*, Dong & de Kroon (1994) ont suggéré que les rhizomes étaient préférentiellement des organes de réserve, tandis que les stolons étaient plutôt impliqués dans la croissance clonale et l'expansion latérale. Néanmoins, Stuefer & Huber (1999) ont montré que la survie de jeunes ramets de *Potentilla anserina*, séparés du genet d'origine, était accrue lorsque ceux-ci étaient connectés à un fragment de stolon. Cette observation, couplée à la quantité importante de cellules parenchymateuses observée dans les entre-nœuds des stolons, suggère donc le rôle potentiel de ces derniers dans le stockage de ressources. Enfin, la base des tiges est également fortement impliquée dans la mise en place de réserves. Ainsi, les espèces consolidatrices (*i.e.*

produisant des connexions très courtes voire inexistantes et des ramets nombreux), notamment les plantes cespiteuses (*i.e.* présentant une croissance en touffe) seraient capables de monopoliser les ressources disponibles localement et ponctuellement, et de les stocker dans la base des tiges de leurs ramets (de Kroon & Schieving 1990, Cheplick & Chui 2001).

1.5. Diversité des formes clonales

La croissance clonale chez les plantes vasculaires peut s'exprimer sous différentes formes. Plusieurs études ont donc eu pour objectif la description, la classification et l'évaluation de la fonction des formes de croissance clonale.

1.5.1. Stratégies clonales

Selon Stearns (1976), une stratégie peut être définie comme « un ensemble de traits co-adaptés permettant à l'organisme de faire face à des problèmes écologiques donnés ». Certaines classifications se sont cependant limitées à ranger les plantes clonales le long de gradients, selon leur degré d'expression d'une propriété clonale donnée.

La **classification phalange – guérilla** (Lovett-Doust 1981) repose sur le fait que l'architecture clonale peut varier de formes très compactes à des formes très dispersées. Dans le premier cas, les connexions ont généralement des entre-nœuds courts et un degré de ramification important. Ces formes de type phalange résultent en une agrégation importante des ramets. Les graminées ou graminoïdes en touffes (formes cespiteuses) représentent un cas de phalange extrême. Les formes de type guérilla possèdent des connexions très peu ramifiées et constituées d'entre-nœuds longs. Les ramets sont dispersés dans l'espace.

La définition de stratégies clonales peut également reposer sur le degré d'intégration. En effet, la durée de connexion entre les ramets et l'individu clonal d'origine (*i.e.* durée d'intégration physique) peut être très variable et peut être classée le long d'un continuum (Oborny *et al.* 2001). La stratégie **séparatrice** ou **désintégratrice** est caractérisée par des connexions éphémères, de durée de vie inférieure à un an. A l'opposé, la stratégie **intégratrice** désigne les individus clonaux chez lesquels les connexions ont une durée de vie de plusieurs années. Parmi les formes intégratrices, la durée de fonctionnalité des connexions (durée d'intégration physiologique) peut varier de moins d'un an (stratégie **intégratrice restrictive**) à plusieurs années (stratégie **intégratrice extensive**).

La prise en compte de traits clonaux sous-jacents à plusieurs propriétés clonales permet une description plus complète des stratégies clonales. Ainsi, de Kroon & Schieving (1990) ont distingué trois stratégies clonales (*foraging*, *conservative*, *consolidation*) sur la

base de traits clonaux relatifs à l'architecture, la durée de vie des connexions, le degré d'intégration physiologique, les capacités de stockage, ainsi que la plasticité de ces traits en réponse à la disponibilité et la distribution spatiale des ressources.

Lors de la prise en compte de plusieurs traits clonaux, il est parfois difficile de déterminer leur réelle implication dans la réponse aux conditions environnementales, certains traits pouvant être corrélés entre eux (Wildová *et al.* 2007). Ainsi, au terme stratégie, nous préfèrerons l'utilisation des termes « *syndromes* » ou « *combinaisons* » de traits.

1.5.2. Classifications qualitatives

Certains auteurs ont cherché à classer les formes clonales en se basant sur leur description morphologique et la caractérisation des différents organes impliqués dans la croissance clonale. Ces démarches peuvent être complétées par la prise en compte de caractères relatifs au degré d'expression d'une propriété donnée (*i.e.* de l'ordre de la stratégie clonale, cf. § ci-dessus)

1.5.2.1. Classification fonctionnelle de Grace (1993)

La démarche conceptuelle de Grace (1993) se concentre sur les plantes aquatiques, chez lesquelles la clonalité est fortement représentée. Elle se base sur la description de syndromes de six fonctions associées à la croissance clonale. Cependant, plus qu'un système de classification, l'objectif de l'auteur est de fournir une comparaison fonctionnelle des différents modes de reproduction clonale, et d'en estimer la valeur adaptative dans diverses conditions environnementales.

1.5.2.2. Typologie des organes de croissance clonale (CGOs)

Cette démarche repose sur la description de traits morphologiques relatifs à la croissance clonale, notamment les organes clonaux (**CGOs**, **Clonal Growth Organs**, Klimeš *et al.* 1997), qui sont les organes à partir desquels se produit la multiplication végétative. Ils peuvent être définis comme les organes « possédant une banque de bourgeons végétatifs et fournissant les connexions vasculaires entre les tiges » (Kleyer *et al.* 2008). La discrimination des différents CGOs se base principalement sur leur origine (racinaire, caulinaire ou foliaire), leur position (aérienne ou souterraine), leur durée de vie et leur fonction de stockage (Klimešovà & Klimeš 2008). Dix-sept types d'organes clonaux ont été distingués (Klimeš *et al.* 1997, Klimešovà & Klimeš 2008, Klimešovà & de Bello 2009 ; Fig. III).

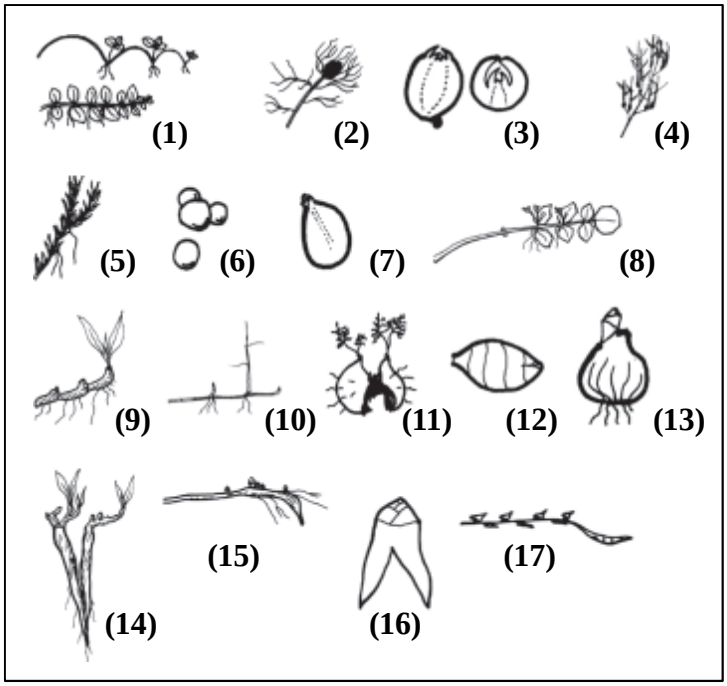

Fig. III. Les 17 types d'organes clonaux (CGOS, Clonal Growth Organs) décrits par Klimesova & Klimes (2008).
(1) Tige plagiotrope aérienne,
(2) Turions,
(3) Bulbes et tubercules d'origine caulinaire, à la surface du sol ou légèrement au-dessus,
(4) Pseudoviviparité,
(5) Fragments de tiges,
(6) Plantes bourgeonnantes,
(7) Tubercules d'origine racinaire, à la surface du sol ou au-dessus,
(8) Bourgeons sur les feuilles,
(9) Rhizomes épigéogènes,
(10) Rhizomes hypogéogènes,
(11) Tubercules se fragmentant;
(12) Tubercules caulinaires, (13) Bulbes,
(14) Racines se fragmentant;
(15) Bourgeons adventifs sur les racines,
(16) Tubercules racinaires souterrains,
(17) Tubercules se développant à l'extrémité distale des tiges aériennes.

Plusieurs études ont utilisé les organes clonaux comme base de leur classification. Ainsi, van Groenendael *et al.* (1996) ont proposé une classification des plantes clonales de la flore d'Europe centrale. Il en émerge quatre groupes de plantes distingués sur la base de la durée de vie et la distance de propagation de leurs organes clonaux. Suite à ce travail, Klimeš *et al.* (1997) ont pris en compte non seulement des informations relatives à la durée de vie et aux capacités d'expansion latérale des organes clonaux, mais aussi au taux de multiplication clonale ou encore à la taille et la position des banques de bourgeons végétatifs. Leur classification aboutit à la description de 21 modes de croissance clonale, de manière à ce qu'une espèce n'appartienne qu'à un seul type (rarement deux ou trois). Enfin, Klimešovà & Klimeš (2007) ont repris les CGOs et les ont classés en quatre catégories, sur la base du type de banque de bourgeons végétatifs qui leur est associée (aérienne *vs*. souterraine et annuelle *vs*. pérenne). Bien que concernant la flore d'Europe centrale, la typologie des CGOs ainsi que les diverses classifications qui en ont été faites peuvent être étendues à d'autres zones au climat tempéré, mais aussi aux régions arctiques et subarctiques (Klimeš *et al.* 1997).

1.6. Ecologie et distribution des plantes clonales

La capacité de croissance clonale est largement répandue dans différents systèmes écologiques, malgré des disparités selon les habitats considérés. Ainsi, les plantes clonales

sont principalement rencontrées dans les milieux humides, pauvres en nutriments ou caractérisés par de faibles températures (van Groenendael *et al.* 1996, Klimeš *et al.* 1997).

Plus précisément, plusieurs études ont montré que les formes de croissance clonale et l'expression des traits clonaux sont dépendants des conditions environnementales, et diffèrent selon les communautés considérées (Klimeš *et al.* 1997, Tamm *et al.* 2002, Sammul *et al.* 2004, Halassy *et al.* 2005, Sosnová *et al.* 2009). L'expression des formes clonales en réponse à des facteurs environnementaux, notamment des perturbations, à moindre échelle spatiale (*e.g.* au sein d'une même communauté) est, quant à elle, moins connue.

Par exemple, une majorité d'espèces herbacées de communautés prairiales présentent la capacité de croissance clonale (Klimeš *et al.* 1997). Cependant, l'impact du mode de gestion de ces prairies (*e.g.* régime de pâturage, fauche, fertilisation, etc.) sur les caractéristiques clonales de la végétation demeure peu connu.

2. Le pâturage

2.1. Impacts du pâturage sur les plantes

2.1.1. Définition du pâturage

Le pâturage par les grands herbivores mammaliens, sauvages ou domestiques, est un facteur biotique complexe s'appliquant aux plantes, et pouvant être décomposé en trois actions majeures : la consommation de la biomasse aérienne, souvent désignée par le terme de **défoliation**, le **piétinement** et la **fertilisation** via les dépôts d'urine et de fèces. Deux types d'effets peuvent être dissociés lorsque le pâturage est considéré à l'échelle de la plante. Les effets **directs** représentent les effets sur la plante elle-même, les effets **indirects** agissent sur l'environnement biotique et abiotique (Hulme 1996).

Malgré l'importance relative de chacun des trois facteurs associés au pâturage, la défoliation émerge comme l'élément principal impactant la végétation (Kolher *et al.* 2004). Par conséquent, la simulation expérimentale du pâturage est souvent simplifiée par l'application d'une défoliation manuelle des plantes, également désignée sous le terme de coupe (*cutting* ou *clipping* en anglais).

2.1.2. Effets directs

La défoliation, mais également le piétinement et, dans une moindre mesure, le dépôt de fèces endommagent les plantes et génèrent la destruction partielle ou totale de tissus aériens

(**perturbation** *sensu* Grime 1977) ou une perte de leur fonctionnalité. Le piétinement peut rompre les tissus. Il est fréquemment cité comme cause principale de la rupture des connexions clonales, générant la fragmentation du genet (Charpentier *et al.* 1998, Fuhlendorf & Smeins 1999). Selon son moment d'occurrence, la défoliation peut être à l'origine de dommages causés aux bourgeons floraux, aux fleurs ou aux fruits, pouvant ainsi limiter l'ampleur de la reproduction sexuée (Bakker *et al.* 1983, Bakker *et al.* 2006).

2.1.3. Effets indirects

Le pâturage affecte également les plantes en modifiant leur environnement biotique et abiotique. Le dépôt de fèces, le piétinement et la défoliation des plantes voisines génèrent l'ouverture de la canopée voire la création de trouée. Il en résulte des modifications des interactions entre plantes, notamment la diminution de la pression compétitive. En particulier, le pâturage a souvent été associé à l'augmentation de la quantité et de la qualité de lumière atteignant le sol (*e.g.* Sala *et al.* 1986, Bakker *et al.* 2003, Veen *et al.* 2008).

L'apport de nutriments via les dépôts d'urine et de fèces se traduit généralement par l'augmentation de la quantité et du taux de minéralisation d'azote dans le sol (*e.g.* Bakker *et al.* 2003, Rossignol *et al.* 2006, Olofsson *et al.* 2008, Veen *et al.* 2008). L'humidité du sol, quant à elle, tend à diminuer en réponse au pâturage, notamment du fait de la compaction générée par le piétinement (Bakker *et al.* 1983, Posse *et al.* 2000), favorisant le ruissellement de l'eau, plutôt que son infiltration (Rietkerk *et al.* 2000).

2.2. Réponses des plantes au pâturage

2.2.1. Stratégies de résistance au pâturage et réponses fonctionnelles

Le pâturage agit comme un filtre environnemental, c'est-à-dire un facteur supprimant les espèces qui ne possèdent pas les traits fonctionnels leur permettant de persister dans les conditions qu'il génère (Diaz *et al.* 1998). Les réponses des plantes au pâturage et à la défoliation qui lui est associée ont été bien étudiées (voir par exemple van der Meijden *et al.* 1988, Juenger & Lennartson 2000, Tiffin 2000), mais la terminologie employée pour les décrire est variée voire conflictuelle, de mêmes termes pouvant avoir des définitions différentes. Nous nous placerons ici dans le cadre défini par Briske (1996) concernant la réponse des plantes à la défoliation liée au pâturage. La **résistance** désigne les mécanismes permettant à la plante de survivre, se développer et se reproduire dans des systèmes pâturés. La résistance au pâturage peut être divisée en deux composantes, définies principalement sur

la base de la réponse de la plante à la défoliation : l'**évitement** correspond aux mécanismes réduisant la probabilité et l'intensité du pâturage s'appliquant à la plante, tandis que la **tolérance** est la capacité de reprise de croissance et de reproduction après pâturage.

2.2.1.1. Evitement

L'évitement peut être constitutif, spatial ou temporel. L'évitement **constitutif**, aussi parfois désigné par le terme de **défense** (de Jong & van der Meijden 2000), repose sur un panel de traits morphologiques et/ou biochimiques, repoussant les herbivores en rendant la plante moins digeste (McIntyre *et al.* 1999). Ainsi, les espèces herbacées peuvent contenir des composés secondaires toxiques tels que les alcaloïdes, les composés phénoliques ou cyanogènes, les tannins ou la silice (Briske 1996, McIntyre *et al.* 1999). La constitution morphologique de la plante peut elle-même constituer un moyen de défense contre les herbivores. La rugosité des feuilles et une forte teneur en tissus de soutien peu digestes, des structures telles que des poils ou des épines permettent l'évitement de la plante par l'herbivore (Briske 1996, McIntyre *et al.* 1999). Ainsi, les espèces les plus digestes sont généralement défavorisées par le pâturage, résultant en une augmentation de la fréquence des plantes les moins palatables (Wardle *et al.* 2001, Diaz *et al.* 2007).

Les mécanismes d'évitement **spatial** portent sur la structure verticale et horizontale de la plante, ainsi que sur la taille des feuilles (Briske 1996), en réduisant l'accessibilité de la biomasse aérienne aux herbivores. Des espèces de petite taille ou à port prostré (notamment les plantes en rosettes), dont les bourgeons floraux et végétatifs, ainsi que les méristèmes actifs sont **près du sol** sont moins accessibles ou moins endommagées par les grands herbivores, (Briske 1996, McIntyre *et al.* 1995, 1999, Bullock *et al.* 2001, Noy-Meir et Briske 2002). En effet, la taille des plantes tend à être plus petite en milieux perturbés (Lavorel *et al.* 1997). En outre, un port prostré et une architecture stolonifère sont favorisés par le pâturage (Diaz *et al.* 2007), tandis que les chamaephytes (dont les bourgeons se situent entre 0 et 25 cm au-dessus du sol), voire les phanérophytes (végétaux de grande taille) sont sensibles au pâturage, certainement du fait de la position de leurs bourgeons les rendant facilement accessibles aux herbivores (McIntyre *et al.* 1995).

L'évitement **temporel** dépend du **cycle phénologique** de la plante : il consiste à passer la période de pâturage sous une forme peu accessible aux herbivores, ou pour laquelle une destruction partielle de la biomasse n'aura pas d'effet délétère important sur la fitness de la plante. Les espèces annuelles éphémères et les plantes thérophytes (*i.e.* passant la majeure

partie de leur cycle phénologique sous forme de graines) ont une probabilité plus faible de subir la perturbation due aux herbivores (McIntyre *et al.* 1995, Briske 1996). C'est aussi le cas d'espèces dont le cycle de vie est décalé par rapport à la saison de pâturage. Certaines plantes terminent leur cycle de vie précocement et leurs parties aériennes dessèchent en été, ne pouvant ainsi être pâturées (Sternberg *et al.* 2000). De plus, passer la saison de pâturage sous forme d'organes tels que des bulbes ou des graines permet à la plante de ne pas être endommagée (Milchunas & Noy-Meir 2002).

2.2.1.2. Tolérance et compensation

La tolérance au pâturage consiste en une **forte reprise de croissance** (Strauss & Agrawal 1999, Bullock *et al.* 2001) et une **reproduction importante** après perturbation (McIntyre *et al.* 1995, 1999). La plupart des travaux relatifs à la reproduction après des perturbations telles que le pâturage se sont focalisés sur la régénération par graines (niche de régénération *sensu* Grubb 1977). A l'inverse, l'intérêt porté à la capacité de **régénération végétative**[6] (niche de persistance, Bond & Midgley 2001) est beaucoup plus récent et s'est notamment accru au cours de la dernière décennie (Klimešovà & Klimeš 2007, Lasso *et al.* 2009). La capacité de régénération végétative a été principalement étudiée chez les plantes ligneuses chez lesquelles elle est très développée (voir par exemple Bellingham & Sparrow 2000, Del Tredici 2001, Bond & Midgley 2001, 2003, Lasso *et al.* 2009), mais elle existe également chez les plantes herbacées (Klimešovà & Klimeš 2003, Klimešovà & Klimeš 2007). Elle aurait un rôle au moins aussi important que la reproduction sexuée et serait notamment plus avantageuse en conditions de perturbations fréquentes et d'intensité modérée (Bellingham & Sparrow 2001, Klimešovà & Klimeš 2003).

La tolérance repose essentiellement sur des caractéristiques morphologiques et physiologiques : présence et activité de méristèmes et de bourgeons régénératifs, notamment basaux (McIntyre *et al.* 1999, Ferraro et Oesterheld 2002), quantité importante de substances de réserve rapidement remobilisables (Strauss & Agrawal 1999), reprise rapide de l'absorption racinaire, augmentation de la SLA[7] (Sala *et al.* 1986, Lattanzi *et al.* 2004) associée à la réinitialisation de l'activité photosynthétique, fort taux de croissance (Tiffin

[6] ***Resprouting*** en anglais. Ce trait a été principalement étudié de manière qualitative, selon la dichotomie des plantes possédant cette capacité (*sprouters* ou *resprouters*) *versus* celles qui ne l'ont pas (*non-sprouters* ou *seeders*).

[7] SLA : *Specific Leaf Area* ou surface foliaire spécifique. Elle correspond au ratio de la surface foliaire par la masse sèche de la feuille.

2000) et plasticité importante au niveau du développement et de l'allocation des ressources (Strauss & Agrawal 1999, Barney *et al.* 2005).

La notion de **compensation** (*sensu* McNaughton 1983) permet de classer le degré de tolérance le long d'un gradient (Maschinski & Whitham 1989, Stowe *et al.* 2000). Lorsque des plantes endommagées survivent et se développent, mais que leurs performances (croissance et reproduction) sont inférieures à celles de plantes non endommagées, la tolérance est incomplète (*undercompensation sensu* Strauss & Agrawal 1999). La tolérance compensatoire, voire sur-compensatoire fait référence au maintien voire à l'augmentation des performances après pâturage (respectivement, *compensation* et *overcompensation*, Stowe *et al.* 2000).

2.2.1.3. Autres mécanismes de résistance au pâturage

La classification des stratégies de résistance au pâturage proposée par Briske (1996) se concentre essentiellement sur les mécanismes d'évitement et de tolérance à la défoliation. Néanmoins, comme nous l'avons vu précédemment, le pâturage est un facteur complexe qui favorise les plantes résistantes ou tirant bénéfice des conditions environnementales qu'il génère.

La **résistance au piétinement** dépend principalement de la hauteur et du type de plantes. Les graminoïdes cespiteuses ou rampantes et, dans une moindre mesure, les plantes en rosette présentent les meilleures capacités de résistance (Cole 1995), certainement car leur tissus de soutien sont moins abondants et moins sévèrement endommagés par le piétinement.

Le pâturage favorise également les traits permettant une **dissémination** et une **colonisation efficace des trouées**. Parmi ces traits, citons un cycle de vie court et une dissémination des graines ou une expansion clonale sur de longues distances (Fahrig *et al.* 1994, McIntyre *et al.* 1995). Les graines et propagules propagés par les herbivores eux-mêmes, soit par l'intermédiaire de leur fourrure (**épizoochorie**, Couvreur *et al.* 2008), soit après passage par leur tube digestif (**endozoochorie**, Moussie *et al.* 2005) sont particulièrement avantageux.

Enfin, la tolérance des plantes au pâturage peut être accrue chez les espèces bénéficiant de la diminution de la pression compétitive et de l'augmentation des ressources lumineuses (ouverture de la canopée) ou des pulses de nutriments via les urines et les fèces.

2.2.2. Réponses à l'échelle de la communauté

2.2.2.1. Diversité et composition floristiques

Le pâturage est généralement considéré comme un facteur à l'origine de l'augmentation de la **diversité spécifique**, notamment en générant des modifications de l'équilibre entre compétition et colonisation (Bakker *et al.* 2006). Ainsi, il est attendu que la consommation d'espèces dominantes diminue l'exclusion compétitive, tandis que l'ouverture de trouées favoriserait la germination et l'établissement d'espèces peu compétitives (Olff & Ritchie 1998). Cependant, plusieurs études ont conclu à des effets contrastés du pâturage sur la diversité (Milchunas *et al.* 1988), allant de l'augmentation (*e.g.* Collins *et al.* 1998, Bakker *et al.* 2003, Kohyani *et al.* 2008) au maintien (*e.g.* Stohlgren *et al.* 1998, Adler *et al.* 2005) voire à la diminution (*e.g.* Wardle *et al.* 2001) de la diversité spécifique en réponse au pâturage.

Les changements de diversité s'accompagnent généralement de modifications de la **composition spécifique**. Les espèces peuvent être classées en fonction de leur réponse au pâturage. Les types *increaser*, *decreaser* et *neutral*, désignent respectivement des espèces dont l'abondance augmente, diminue ou reste stable, respectivement, en conditions pâturées par rapport aux conditions non pâturées (Dykserhuis 1949, Diaz *et al.* 2001, Vesk & Westoby 2001, del-Val & Crawley 2004, 2005). Une même espèce peut néanmoins présenter des réponses contrastées en fonction des caractéristiques du site étudié (Vesk & Westoby 2001). En effet, de nombreux facteurs modulent l'impact du pâturage sur les plantes, soit parce qu'ils agissent comme des filtres principaux, favorisant l'expression de certains traits, soit parce qu'ils influencent la réponse des plantes soumises au pâturage.

2.2.2.2. Diversité fonctionnelle

Comme nous venons de le voir, la réponse des plantes au pâturage est généralement associée à l'expression de traits fonctionnels. Sur la base de ces traits, les espèces présentant des réponses au pâturage similaires peuvent être regroupées en groupes fonctionnels de réponse (Lavorel & Garnier 2002). Ainsi, l'impact du pâturage sur les communautés végétales peut être estimé en termes, non plus de diversité spécifique, mais de diversité fonctionnelle, définie comme l'amplitude de différence de l'expression de traits au sein de la communauté (Tilman 2001). Bien que les impacts du pâturage sur les traits fonctionnels soit une thématique largement explorée, les réponses au pâturage en termes de diversité fonctionnelle demeurent moins connues. Il semblerait cependant que les impacts du pâturage sur la diversité

fonctionnelle soient variables, et peu dépendants de la diversité spécifique (de Bello *et al*. 2006).

2.2.3. Facteurs modulant l'impact du pâturage sur les plantes et les communautés

De nombreux facteurs environnementaux filtrent le pool d'espèces et de traits (Diaz *et al.* 1998). L'étude des traits fonctionnels de réponse au pâturage doit prendre en compte ces divers filtres, et ne peut généralement s'appliquer qu'à échelle régionale (de Bello *et al.* 2005, Diaz *et al.*2007). Parmi ces facteurs, le **contexte climatique** est filtre important. Par exemple, des climats secs ou subalpins et le pâturage tendent à favoriser les mêmes traits. La **productivité** du milieu a souvent été suggérée comme un facteur modulant les effets du pâturage sur la diversité et la composition spécifiques (Pakeman 2004, Olff & Ritchie 1998, Bakker *et al*. 2006, de Bello *et al.* 2006). Ainsi, l'augmentation de la diversité spécifique en réponse au pâturage s'exercerait principalement dans les milieux productifs, caractérisés par des forts taux de croissance et des processus d'exclusion compétitive rapides, en l'absence de contrôle par les herbivores (de Bello *et al.* 2006, Marion *et al*. submitted). Les impacts du pâturage sur la végétation sont également modulés par le régime d'inondation (Chaneton & Facelli 1991, Oesterheld & McNaughton 1991, Insausti *et al.* 1999, Jutila 1999).

La réponse des plantes au régime de pâturage est également contrainte par des facteurs intrinsèques au pâturage lui-même, tels que son historique (Milchunas *et al.* 1988, Diaz *et al.* 2007), la **saison** où il est appliqué, son intensité (régime de pâturage), le **type** et la **taille des herbivores** (Bullock *et al.* 2001). Ainsi, le taux de mortalité (Bullock *et al.* 1994) ainsi que les capacités de reprise de croissance et de reproduction (Sternberg *et al.* 2000, Garcia & Ehrlen 2002) sont différemment affectés selon le **stade phénologique** de la plante au moment de la perturbation. D'autre part, les herbivores de grande taille (*e.g.* bovins) seraient souvent associés à l'augmentation de la diversité spécifique, par leur comportement alimentaire peu sélectif et leur impact sur les espèces dominantes (Bakker *et al.* 2006, de Bello *et al.* 2006). Néanmoins, la sélectivité des herbivores dépend de la quantité de biomasse qui leur est accessible ainsi que du pool d'espèces présentes, et de leur palatabilité relative (Bakker *et al.* 1983, Fuhlendorf & Smeins 1999). En conditions intensives, une partie importante de la végétation est consommée. Ainsi, en ne permettant qu'aux espèces les plus tolérantes de se développer, un pâturage intensif peut générer une diminution de la diversité (Olff & Ritchie 1998).

Enfin, de nombreuses études ont montré que le pâturage agit sur l'hétérogénéité des conditions environnementales et de la végétation, dépendamment des facteurs cités

précédemment, mais aussi de l'**échelle** considérée. L'échelle d'observation constitue donc un élément crucial à l'origine des variations de la réponse des communautés végétales au pâturage (Fuhlendorf & Smeins 1999, Adler *et al.* 2001, Marion *et al*. in prep. a, b).

2.3. Pâturage et hétérogénéité spatio-temporelle

Le pâturage ne s'applique pas de manière homogène à la végétation. Le dépôt de fèces et d'urine (Olff & Ritchie 1998), l'ouverture de la canopée et la création de trouées par piétinement ou défoliation, ou encore la consommation préférentielle de certaines plantes et l'existence de zones de refus (Milchunas & Noy-Meir 2002) sont autant d'éléments potentiellement générateurs d'hétérogénéité spatiale (Adler *et al.* 2001), tant du point de vue des conditions abiotiques (*e.g.* compaction du sol, quantité de nutriments, micro-climat) pouvant s'exprimer à différentes échelles (Rietkerk *et al.* 2000), que de la structure de la végétation (*e.g.* hauteur de la canopée, composition spécifique, distribution horizontale des espèces).

2.3.1. Différentes échelles d'hétérogénéité

De nombreux travaux ont étudié les conséquences du pâturage sur les composantes biotiques et abiotiques des prairies et leur échelle d'expression. Il en ressort que le pâturage s'applique à diverses échelles (Adler *et al.* 2001, Veen *et al.* 2008). Plusieurs échelles peuvent être prises en compte lors de l'étude du comportement des herbivores. Par exemple, la défoliation a lieu à l'échelle de la bouchée (Schwinning & Parsons 1999) mais peut s'étendre sur une surface plus importante par de simples mouvements de tête (échelle fine) puis, par déplacement, à l'échelle de la station d'alimentation (*feeding site*) voire entre patches de végétation. A des échelles spatio-temporelles plus importantes, la défoliation peut s'exprimer différemment entre des communautés différentes (WallisDeVries *et al.* 1999). La défoliation tend à être orientée sur les espèces les plus palatables. Cependant, plusieurs études ont suggéré que la sélectivité des grands herbivores mammaliens ne pouvait pas s'exprimer à échelle fine, du fait de leur grande taille. Ainsi, WallisDeVries *et al.* (1999) ont montré que les herbivores sélectionnaient les patches de meilleure qualité nutritionnelle, mais que la défoliation à l'intérieur de ces patches était un évènement probabiliste. En d'autres termes, la défoliation à échelle fine (de la bouchée et de la station d'alimentation) serait aléatoire et génératrice d'hétérogénéité intra-patch (Schwinning & Parsons 1999, Adler *et al.* 2001).

En parallèle, les dépôts de fèces et d'urine, s'appliquent de manière localisée, créant des micro-conditions de quelques dizaines de centimètres (Augustine & Frank 2001).

Cependant, leurs effets sont parfois plus étendus, notamment en fonction du comportement des herbivores, qui peuvent déposer urine et fèces dans des zones de position fixe (*e.g.* latrines dans le cas de pâturage équin, Loucougaray *et al.* 2004). Enfin, d'autres comportements tels que le piétinement ou le repos sont également générateurs d'hétérogénéité à diverses échelles (Oom *et al.* 2008).

Le pâturage modifie le milieu abiotique et la végétation à diverses échelles. De nombreuses études ont démontré la distribution hétérogène des ressources édaphiques et des flux de nutriments dans des écosystèmes variés (Augustine & Frank 2001 et références citées). Cette hétérogénéité dépend non seulement des conditions abiotiques et floristiques locales (*e.g.* topographie, climat, composition floristique, Rietkerk *et al.* 2000, Augustine & Frank 2001), mais peuvt également être générés ou modulés par le pâturage. Comme nous l'avons vu précédemment, les dépôts de fèces et d'urine génèrent le plus souvent des apports d'azote et une augmentation du taux de minéralisation. La modification des patrons de distribution spatiale des ressources édaphiques par le pâturage a été démontrée à maintes reprises. L'hétérogénéité résultante s'exprime à diverses échelles, de quelques centimètres (Augustine & Frank 2001), à quelques mètres voire plusieurs dizaines de mètres (Augustine & Frank 2001, Rossignol *et al.* 2006, Olofsson *et al.* 2008, Zhou *et al.* 2008). A contrario, certains travaux ont montré le maintien de la distribution des ressources édaphiques à des échelles comparables (Bakker *et al.* 2003, Veen *et al.* 2008), suggérant des réactions différentes en fonction des conditions d'étude (site d'étude, type et nombre d'herbivores).

L'hétérogénéité de la structure du sol, sa teneur en eau et en éléments ioniques sont également modifiées par le pâturage, principalement via la compaction du sol liée au piétinement (Posse *et al.* 2000, Rietkerk *et al.* 2000).

Le pâturage modifie également la structure de la végétation et augmente la fréquence des sites ouverts (Weber *et al.* 1998). En lien avec les patrons spatiaux de la défoliation et du piétinement, la présence de sol nu et la structure verticale (hauteur) de la végétation, ainsi que la distribution horizontale des ressources lumineuses et de la température du sol qui en résultent (quantité de lumière atteignant le sol) sont généralement hétérogènes (de quelques centimètres à plusieurs mètres, Bakker *et al.* 1983, Rietkerk *et al.* 2000, Augustine 2003, Bakker *et al.* 2003, Olofsson *et al.* 2008).

2.3.2. Composition de la végétation

Les modifications de l'hétérogénéité des ressources édaphiques ainsi que de la structure verticale de la végétation (hauteur de la canopée, présence de sol nu) et des ressources

lumineuses se traduisent généralement par des changements de la composition spécifique à diverses échelles. Dans une étude relativement ancienne, Bakker *et al.* (1983) ont observé cette structuration de la végétation selon deux niveaux emboîtés : inter-communauté ('macro-pattern') et intra-communauté (0.5-3 m, 'micro-pattern'). Cependant, bien que la structure de la végétation soit hétérogène à ces deux échelles, la composition floristique, quant à elle, ne s'est avérée variable qu'à l'échelle la plus large. La formation de patches de végétation de composition différente et de quelques mètres à quelques centaines de mètres de diamètre, a été régulièrement reportée (Posse *et al.* 2000, Adler *et al.* 2001, Augustine 2003, Loucougaray *et al.* 2004, Oom *et al.*2008).

L'impact du pâturage sur l'hétérogénéité des conditions environnementales et de la composition de la végétation explique, dans certains cas, l'augmentation de la richesse et de la diversité spécifique observée en prairies pâturées (Bakker *et al.* 2003, Anderson *et al.* 2004, Zhou *et al.* 2008, Olofsson *et al.* 2008).

2.3.3. Feedbacks entre pâturage, conditions environnementales et végétation

Bien que s'appliquant de manière hétérogène sur l'environnement abiotique et sur la végétation, le pâturage n'est pas toujours créateur d'hétérogénéité au sein de cette végétation. Il a même parfois été montré que le pâturage tendait à l'homogénéisation du couvert végétal (Adler *et al.* 2001, Collins & Smith 2006). En effet, l'impact effectif du pâturage sur l'hétérogénéité de la végétation dépend de l'interaction entre leurs patrons spatiaux respectifs (Adler *et al.* 2001). En particulier, l'existence de feedbacks entre le pâturage, les conditions environnementales qu'il génère et la composition de la végétation joue un rôle crucial dans la création et la préservation des patrons spatiaux de la végétation en prairies pâturées.

La sélectivité des herbivores à l'échelle du patch implique que les caractéristiques de la végétation, notamment sa composition et son hétérogénéité intrinsèque, orientent le pâturage vers les patches les plus palatables, et influencent ainsi son patron spatial (Bakker *et al.* 1983, Loucougaray *et al.* 2004). Les capacités de reprise de croissance de la végétation de bonne qualité nutritionnelle après pâturage (compensation) conditionnent donc l'existence de feedbacks et leur direction (positive ou négative) entre pâturage et qualité de la végétation. Si le pâturage diminue la quantité de tissus sénescents et augmente la quantité et la qualité de la végétation (*e.g.* teneur en protéines plus importante dans les jeunes feuilles), les zones préférentiellement pâturées seront les mêmes (forte prédictibilité spatio-temporelle du patron spatial de la végétation et du pâturage). Dans ce cas (feedback positif), le pâturage tend à maintenir, voir à augmenter, l'hétérogénéité de la végétation à l'échelle du patch (Adler *et al.*

2001, Mouissie *et al.* 2008). A l'inverse, par des feedbacks négatifs, par exemple dus à la sélection de traits d'évitement (*e.g.* petite taille, substance de défense), le pâturage tendra à l'homogénéisation du couvert végétal (Adler *et al.* 2001).

Le pâturage influence également la composition de la végétation de par son impact sur l'hétérogénéité des conditions abiotiques. Les caractéristiques de la végétation (structure, composition floristique) peuvent à leur tour modifier les conditions abiotiques (*e.g.* infiltration de l'eau, cycle de l'azote, Rossignol *et al.* 2006) et biotiques (*e.g.* interactions compétitives). Par conséquent, le pâturage peut structurer la végétation de manière indirecte, par cette boucle d'auto-organisation des patrons de végétation ('*self-organization*', Alados *et al*. 2007).

3. Questions, hypothèses et axes de travail

3.1. Clonalité et réponse au pâturage

Bien que les études cherchant à caractériser les traits fonctionnels de réponse au pâturage soient nombreuses, la clonalité y est rarement incluse ou elle y est considérée comme un trait elle-même (Weiher *et al.* 1999, Klimešovà & de Bello 2009). Seules quelques informations sur les formes de croissances clonales rencontrées en milieux perturbés par la défoliation, qu'elle soit due au pâturage ou à la fauche, sont disponibles. Diaz *et al.* (2007) ont montré que le pâturage tendait à favoriser les formes stolonifères, tandis que les graminées rhizomateuses ou stolonifères apparaissent plus abondantes dans les prairies avec une longue histoire évolutive de pâturage (Mack & Thompson 1982). D'un autre côté, plusieurs études ont montré la dominance de formes clonales à faibles capacités de propagation dans des prairies ouvertes, notamment par la fauche (Klimešová *et al.* 2008).

De par les propriétés singulières qui lui sont liées et leur expression variable, la clonalité pourrait cependant jouer un rôle majeur dans les mécanismes de résistance au pâturage et, plus particulièrement à la défoliation qu'il génère. Les propriétés clonales et les traits clonaux qui les sous-tendent pourraient être impliqués dans différents mécanismes de résistance au pâturage, qu'ils soient associés aux stratégies d'évitement ou de tolérance. L'expression des traits clonaux pourrait notamment dépendre des caractéristiques de la défoliation (Tableau I).

3.1.1. Clonalité et mécanismes de résistance au pâturage

La présence d'une **banque de bourgeons végétatifs stockée au niveau des organes clonaux** aurait deux avantages en prairies pâturées. La position **souterraine ou proche de la surface**

du sol des organes clonaux les rend difficilement accessibles par les herbivores mammaliens (évitement spatial). Les bourgeons végétatifs non endommagés peuvent intervenir dans la tolérance de la défoliation en permettant la multiplication végétative après pâturage (Klimešovà & Klimeš 2003, 2007).

Outre leur fonction de stockage de méristèmes, les organes clonaux peuvent **stocker des quantités importantes de ressources**, directement mobilisables au profit de la croissance compensatoire et de la régénération végétative après perturbation (Bell & Ojeda 1999, Klimeš & Klimešovà 2002).

3.1.2. Le cas particulier de l'hétérogénéité spatiale de la défoliation

La défoliation et le piétinement pouvant endommager les fleurs et les graines, la **propagation clonale sur de longues distances** pourrait permettre la colonisation des trouées et des zones de sol nu ouvertes par le pâturage de manière plus efficace que la propagation sexuée (Fahrig *et al.* 1994). L'**intégration clonale** ainsi que la **plasticité intra-genet** pourrait générer des réponses efficaces à l'hétérogénéité spatio-temporelle de la défoliation induite par le pâturage. L'intégration physiologique permet aux ramets intacts de soutenir la croissance compensatoire des ramets défoliés (Jónsdóttir & Callaghan 1989, Hutchings 1999) et de moyenner l'impact de la défoliation à l'échelle du genet (Hartnett 1989). La défoliation pourrait également induire une spécialisation dans les fonctions de reproduction, les ramets intacts se reproduisant par voie sexuée et les ramets endommagés par voie clonale.

3.2. Axes de travail

L'objectif principal de ce travail de thèse est de chercher à déterminer dans quelle mesure les traits clonaux sont impliqués dans la réponse des plantes au pâturage. Plus particulièrement, nous avons cherché à tester l'hypothèse que **le pâturage favorise les combinaisons de traits clonaux qui confèrent aux plantes des capacités de résistance, notamment à la défoliation qu'il génère**. Dans cet objectif, plusieurs axes de recherche ont été abordés, et seront présentés dans le présent manuscrit. Les questions et hypothèses sont précisées en introduction de chaque chapitre.

La première partie a pour objectif d'évaluer l'influence du pâturage sur les traits clonaux à l'échelle de la communauté. En nous intéressant à des gradients croisés de pâturage et d'inondation, nous avons cherché à déterminer l'influence du pâturage sur les traits clonaux, et notamment dans quelle mesure cet impact peut moduler ou être modulé par un autre facteur environnemental. Nous avons ensuite cherché à caractériser le lien entre

différents critères descriptifs de la défoliation générée par le pâturage (intensité, hétérogénéité spatiale, variabilité temporelle) et les traits clonaux. Enfin, notre objectif a été de déterminer l'implication relative des traits clonaux et de leur réponse à la défoliation dans les patrons d'abondance d'espèces clonales en prairies pâturées. Cette première partie repose sur des données récoltées *in situ*, dans la base de données CLO-PLA3 (Klimešová & Klimeš 2008) et expérimentalement.

La seconde partie se focalise sur la réponse de l'architecture clonale à la défoliation. Les questions posées étaient les suivantes : existe-t-il une ou plusieurs réponses de l'architecture clonale à la défoliation ? La réponse architecturale à la défoliation est-elle dépendante des traits clonaux des espèces ? A savoir la réponse architecturale d'une espèce à la défoliation est-elle dépendante de ses caractéristiques spécifiques, *i.e.* le type d'organe(s) clonal(aux) produit(s) ou les contraintes structurelles constitutives ? Cette seconde partie repose sur deux expérimentations réalisées en serre et en jardin.

La troisième partie aborde les réponses physiologiques des plantes clonales au pâturage. Elle se concentre principalement sur la capacité de mise en place et de remobilisation des réserves stockées dans la base des tiges et cherche à déterminer son rôle dans la résistance au pâturage. Cette troisième partie est constituée d'une revue bibliographique et d'une expérience de laboratoire.

Enfin, la quatrième partie cherche à démêler les rôles respectifs des traits architecturaux et des traits physiologiques (intégration physiologique et stockage de réserves) dans la réponse de plantes clonales à divers patrons de défoliation. Cet objectif est abordé de manière à identifier les liens entre les traits clonaux et les caractéristiques de la défoliation. Ainsi, plusieurs composantes de la défoliation (pourcentage, grain, fréquence et intensité) ont pu être testées et mises en relation avec les combinaisons de traits clonaux dont elles favorisent l'expression. Cette dernière partie repose sur des données de modélisation numérique.

Tableau I – Hypothèses relatives au rôle des traits clonaux dans la résistance à la défoliation induite par le pâturage. Seuls les traits impliqués dans la résistance aux pertes de tissus générés par la défoliation (effets directs) sont pris en compte.

	Mécanisme de résistance	Traits communément associés	Traits clonaux attendus
Défoliation homogène	**Evitement**		
	Défenses	- Composés secondaires toxiques - Constitution morphologique	/
	Evitement spatial	- Petite taille, port prostré, port en rosette - Bourgeons et méristèmes proches du sol	- Organes clonaux porteur d'une banque de bourgeons souterraine (bulbes, tubercules, racines, rhizomes) ou proche de la surface du sol (stolons rampants)
	Evitement temporel	- Décalage phénologique - Formes de résistance (graines)	- Ramets annuels à développement précoce ou automnal - Organes clonaux pérennes
	Tolérance		
	Croissance compensatoire	- Fort taux de croissance - Substances de réserves	- Organes de stockage : bulbes, tubercules, rhizomes (principalement), stolons (dans une moindre mesure) - Stockage dans la base des tiges
	Régénération végétative	- Bourgeons et méristèmes végétatifs actifs	- Fort taux de multiplication clonale - Fort taux de ramification (architecture de type phalange)
Défoliation hétérogène	**Evitement spatial**		- Connexions et distance inter-ramets longues (architecture de type guérilla)
	Tolérance		- Intégration physiologique extensive - Spécialisation des ramets non défoliés dans l'acquisition des ressources

SITE D'ÉTUDE ET MÉTHODOLOGIE GÉNÉRALE

1. Le Marais poitevin, un site d'intérêts écologique et patrimonial remarquables

Le Marais poitevin est la deuxième plus grande zone humide du territoire français et la plus importante du littoral atlantique. Il s'étend sur 120 000 hectares répartis sur trois départements : la Vendée, la Charente-Maritime et les Deux-Sèvres (46°30' - 46°15' Nord et 1°30' – 0°35' Ouest). Le climat y est de type thermo-atlantique à déficit hydrique estival.

Cette zone humide est constituée de terres gagnées sur la mer par des travaux de poldérisation entrepris très vraisemblablement dès le X^{ème} siècle. Elle recèle une diversité rare. Elle est le lieu de passage et de séjour d'oiseaux migrateurs (Grue cendrée, Oie cendrée) et abrite également de nombreux oiseaux nicheurs (Guifette noire, Sarcelle d'été, Chevalier guignette, Chevalier gambette, Barge à queue noire, Combattant varié) ainsi que d'autres espèces animales telles que la Loutre d'Europe ou le Pélodyte ponctué. La végétation y est diversifiée et comprend certaines espèces protégées telles que *Ranunculus ofioglossifolius*. Une telle richesse biologique est liée aux caractères originaux de ces prairies naturelles humides, notamment leur micro-relief et leur gestion pastorale singuliers.

1.1. Micro-topographie, sol et végétation

Les prairies humides sont divisées en trois ensembles, issus du dénivelé de 0.3 à 0.7 m associé à une dynamique hydrique, des caractéristiques pédologiques et une végétation particulière. Les **dépressions inondables** (**baisses**) sont inondées de quatre à six mois par an, en fonction de la pluviométrie et de la gestion hydrique, conduisant à l'expression d'une flore **hygrophile** (*e.g. Glyceria fluitans*, *Alopecurus geniculatus*, *Eleocharis palustris*, *Oenanthe fistulosa*, *Trifolium fragiferum*). Les **replats** ne sont jamais inondés et présentent une végétation **mésophile** (*e.g. Lolium perenne*, *Gaudinia fragilis*, *Cynosurus cristatus*, *Elytrigia repens*, *Hordeum secalinum*). Entre les deux, les **pentes** sont caractérisées par des inondations ponctuelles, mais surtout par une forte humidité estivale (« bourrelet humide ») et des remontées capillaires générant une salinité surfacique importante. Ces particularités favorisent l'existence d'une communauté végétale **méso-hygrophile** et **halophile** (*Juncus gerardii*, *Alopecurus bulbosus*, *Parapholis strigosa*, *Hordeum maritimum*, *Plantago coronopus*).

Le sol y est peu évolué, et caractérisé par son hydromorphie importante. Trois horizons peuvent être distingués : le mat racinaire fibreux (horizon OL, de 5 à 15cm), l'horizon A1g constitué d'un mélange de matière organique et d'une forte proportion d'argile (jusqu'à 30cm de profondeur) et l'horizon Cg très argileux (Bouzillé 1992, Loucougaray 2003).

(a)

(b)

Fig. IV. Vue aérienne (a) et représentation schématique (b) du dispositif expérimental des Magnils-Reigners. B : pâturage bovin, E, pâturage équin, P : pâturage plurispécifique (bovin – équin), TNP : traitement non pâturé (exclos). Les chiffres représentent la charge, en nombre d'animaux par parcelle.

Ce sol présente une salinité et une sodicité singulières et variables selon le niveau topographique : faibles au niveau des dépressions inondables, intermédiaires au niveau des replats et maximales sur les pentes. Ces variations seraient dues à des différences dans la durée d'inondation, mais également dans l'expression des phénomènes de percolation (importante au niveau des dépressions et des replats), de ruissellement et de remontées capillaires (au niveau des pentes).

Les caractéristiques pédologiques (*e.g.* salinité et sodicité, cycles des nutriments) ainsi que la composition floristique sont également modulées par le pâturage, mode de gestion ancestral de ces prairies naturelles (Bouzillé & Tournade 1990, Loucougaray 2003, Rossignol 2006).

1.2. Un mode de gestion ancestral : le pâturage communal

Depuis leur création au X^{ème} siècle, les prairies humides sont exploitées par pâturage libre extensif, auquel elles doivent leur appellation traditionnelle de « marais communaux ». Dès le Moyen-âge, les abbayes et seigneuries y autorisaient les paysans à faire paître vaches, chevaux et oies. Ce mode de pâturage traditionnel a été conservé depuis lors. Aujourd'hui, moyennant une taxe de pâturage, les éleveurs peuvent y installer leurs troupeaux d'avril à décembre. Seize communaux d'une superficie de 15 à 294 hectares sont soumis à ce pâturage pluri-spécifique.

1.3. Le dispositif expérimental des Magnils-Reigners

Le communal des Magnils-Reigners (46°26'20" Nord – 1°12'12" Ouest) est le troisième par sa superficie (234 hectares). Au nord de ce communal, un dispositif expérimental d'environ 20 hectares, permettant de contrôler le type et le chargement en herbivores, a été installé en 1995 (Amiaud 1998). Ce dispositif est composé de 11 parcelles : cinq enclos de 1 hectare pâturés par des bovins, trois enclos de 2 hectares soumis à pâturage équin, deux enclos plurispécifiques (pâturage bovin et équin) de 2 hectares et un exclos sans pâturage (Fig. IV). Au moment de l'installation du dispositif expérimental, les proportions des communautés végétales au sein de chaque parcelle étaient les suivantes : 35-45 % pour la communauté hygrophile, 10-15% pour la communauté méso-hygrophile et 45-55 % pour la communauté mésophile (Amiaud 1998).

2. Méthodes utilisées

L'objectif de cette thèse était de déterminer le rôle des stratégies clonales dans la réponse des plantes au pâturage et, plus particulièrement, à la défoliation qu'il génère. Pour cela, plusieurs outils méthodologiques ont été utilisés (Tableau II).

Les relevés floristiques, les mesures relatives à la structure de la végétation et les prélèvements d'espèces ont été réalisés au sein du dispositif expérimental des Magnils-Reigners. Afin de limiter les dommages causés au sein du dispositif et de ne pas entraver les études ultérieures, les collectes de plantes pour les cultures expérimentales ont eu lieu dans le communal des Magnils-Reigners.

Tableau II. Présentation des outils méthodologiques utilisés au cours de cette thèse.

	Méthodes appliquées et outils utilisés
Chapitre I – Caractérisation des stratégies clonales en réponse aux conditions environnementales	
Article 1	- Base de données Bouzillé J.-B. et Bonis A. : relevés floristiques - Base de données CLO-PLA3 (Klimešovà & Klimeš 2008, Klimešovà & de Bello 2009), d'accès gratuit sur Internet : traits clonaux spécifiques
Article 2	- Mesures *in situ* : relevés floristiques - Mesures *in situ* : structure spatiale de la végétation - Base de données CLO-PLA3 (Klimešovà & Klimeš 2008, Klimešovà & de Bello 2009), d'accès gratuit sur Internet : traits clonaux spécifiques
Article 3	- Mesures *in situ* : relevés floristiques - Expérimentation en jardin : réponse des traits clonaux à la défoliation, 8 espèces
Chapitre II – Réponses morphologiques et architecturales des plantes clonales à la défoliation	
Article 4	- Expérimentation en serre : réponse de traits clonaux à la défoliation, 10 espèces
Article 5	- Expérimentation en jardin : réponse de traits clonaux à la défoliation, 2 espèces
Chapitre III – Réponses physiologiques des plantes clonales à la défoliation et au pâturage	
Article 6	- Prélèvements de plantes *in situ*, 3 espèces - Expérimentation en laboratoire : dosages des réserves carbonées
Article 7	- Prélèvements de plantes *in situ*, 6 espèces - Expérimentation en laboratoire : dosages des réserves carbonées
Chapitre IV – Importance relative des traits architecturaux et physiologiques dans la réponse à la défoliation	
Article 8	- Modélisation : modèle individu centré (IBM : Individual-Based Model)

Les dispositifs expérimentaux et outils utilisés, ainsi que les méthodes d'analyses des données sont présentés en détail dans chaque article.

CHAPITRE 1 – LES TRAITS CLONAUX ET LEUR REPONSE A LA DEFOLIATION SONT-ILS DE BONS INDICATEURS DE LA REPONSE DES PLANTES AU PATURAGE ?

Introduction du chapitre 1

Le pâturage est l'un des principaux modes de gestion des écosystèmes terrestres par l'Homme. Ce phénomène complexe agit sur les plantes à la fois directement, notamment en causant la destruction de certains tissus, et indirectement, en modifiant leur environnement abiotique (*e.g.* disponibilité en ressources, structure du sol) et biotique (*e.g.* interactions plante – plante). De ce fait, le pâturage influence la structure ainsi que la composition spécifique et fonctionnelle de la végétation. En particulier, il peut être générateur d'hétérogénéité spatiale à plusieurs échelles, de quelques centimètres à plusieurs centaines de mètres. Ces effets sont cependant modulés par divers facteurs. Ainsi, de nombreuses études ont souligné la difficulté de décrire les impacts du pâturage à l'échelle globale, du fait de l'influence des caractéristiques propres au site étudié. Par exemple, le climat, la productivité, le régime d'inondation ou encore l'historique de pâturage sont autant de filtres primaires contraignant le pool d'espèces et de traits fonctionnels et conditionnant les réponses de la végétation au pâturage. Les effets du pâturage dépendent également de ses caractéristiques intrinsèques (*e.g.* type, nombre d'herbivores).

Deux stratégies de résistance au pâturage ont été distinguées : l'évitement et la tolérance. L'évitement repose sur des mécanismes permettant de diminuer la probabilité d'être pâturé. Il peut être lié à des traits de défense, repoussant les herbivores (*e.g.* épines, composés toxiques). L'évitement peut également être temporel et consister en des décalages entre le cycle phénologique de la plante et la saison de pâturage, ou spatial, limitant l'accessibilité de la plante aux herbivores. La tolérance repose sur des mécanismes augmentant la survie et amplifiant la reprise de croissance et la reproduction notamment suite à la défoliation générée lors du pâturage.

Les traits impliqués dans les mécanismes d'évitement et de tolérance sont divers. L'impact du pâturage sur la composition fonctionnelle de la végétation fait l'objet de nombreuses études, mais rares sont celles qui s'intéressent à la clonalité et ce, malgré l'abondance des plantes clonales dans la matrice de la végétation prairiale. Dans les prairies humides, telles que les prairies communales du Marais Poitevin, la végétation est soumise à divers régimes de pâturage et d'inondation. L'objectif de ce premier chapitre est de décrire l'influence de divers régimes de pâturage bovin, et plus particulièrement l'impact des pertes

de tissus aériens qu'ils génèrent chez les plantes (défoliation), sur les stratégies clonales exprimées par la végétation. Nous avons cherché à répondre aux questions suivantes :

1- Les régimes de pâturage et d'inondation agissent-ils de manière indépendante ou interactive sur la composition clonale de la végétation (ARTICLE 1) ? En particulier, nous avons émis deux hypothèses :
    a. Ces deux facteurs agissent comme des filtres sélectionnant les traits clonaux impliqués dans la résistance aux conditions qu'ils génèrent
    b. Ces filtres n'impactent pas les traits clonaux de manière indépendante mais, au contraire, leur effets de filtres interagissent entre eux.
2- Le régime de pâturage est-il générateur d'hétérogénéité spatiale de la structure de la végétation ? Plus précisément, la défoliation s'applique-t-elle de manière hétérogène à la végétation, et à quelle échelle ? Cette hétérogénéité est-elle perceptible à l'échelle de la plante clonale ? A-t-elle une influence sur l'expression des traits clonaux (ARTICLE 2) ? Nous sommes partis du principe que la défoliation s'appliquant à une échelle supérieure à celle de la plante clonale (supérieure à 1 mètre), est perçue comme homogène tandis qu'en deçà (entre 10 centimètres et 1 mètre) elle est perçue comme hétérogène. Nous avons émis les hypothèses suivantes :
    a. La défoliation homogène (*coarse-grained*) ne permet pas à la plante de fuir les zones défoliées et favorise donc les traits clonaux associés à des stratégies de tolérance (fort taux de multiplication clonale, organes clonaux spécialisés dans le stockage de ressources).
    b. La défoliation hétérogène (*fine-grained*) favorise les traits clonaux associé à des stratégies d'évitement horizontal (fuite des zones défoliées) avec partage du risque de défoliation entre les ramets (expansion et intégration clonales extensives, *i.e.* connexions et durée d'intégration longues).
3- Les traits clonaux sont-ils de bons indicateurs de la résistance des espèces au pâturage ? La résistance des espèces au pâturage est-elle associée à la réponse des traits clonaux à la défoliation (ARTICLE 3) ? Les hypothèses suivantes ont été testées :
    a. Les espèces les plus résistantes dont l'abondance augmente avec le régime de pâturage, présentent des traits clonaux associés aux stratégies de tolérance et/ou d'évitement. Les espèces les moins résistantes dont l'abondance diminue avec un régime de pâturage croissant, ont des traits leur conférant une forte aptitude compétitive.

b. Chez les espèces les plus résistantes au pâturage, la réponse des traits clonaux à la défoliation augmente la performance clonale (tolérance à la défoliation). Chez les espèces les moins résistantes au pâturage la réponse des traits clonaux à la défoliation diminue la performance clonale (sensibilité à la défoliation).

Pour répondre aux questions 1 et 2 (ARTICLES 1 et 2), nous avons couplé des données issues de mesures *in situ* avec des informations relatives aux traits clonaux collectées dans la base de données CLO-PLA3 (Klimešová & Klimeš 2008). L'étude présentée dans l'ARTICLE 2, a été réalisée sur la végétation mésophile, *i.e.* la moins contrainte par le régime d'inondation. Pour répondre à la question 3 (ARTICLE 3), nous avons sélectionné les huit espèces clonales pérennes les plus abondantes dans la communauté mésophile, que nous avons cultivées en jardin expérimental et soumises à une défoliation expérimentale.

Article 1 – Clonal strategies along flooding and grazing gradients: from database to field patterns

Marie-Lise Benot, Cendrine Mony, Amandine Merlin, Benoit Marion, Jan-Bernard Bouzillé & Anne Bonis

UMR CNRS 6553, ECOBIO, Université de Rennes 1, Campus de Beaulieu, 35042 Rennes Cedex, France

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Abstract

The specific composition and the species' clonal traits were characterized along combined flooding and grazing gradients in order to answer two questions. (1) To which extent does the combination of flooding and grazing influence the clonal characteristics of the vegetation? (2) Are the effects of both environmental factors independent or interactive? This study was carried out in a wet meadow along the Atlantic coast (France). Environmental gradients consisted of three flooding modalities, which discriminated three plant communities (hygrophilous, meso-hygrophilous and mesophilous) and five grazing pressures controlled through an experimental design (from no grazing to heavy grazing). We extracted the type of clonal growth organs (CGOs) and the clonal traits from the free database *CLO-PLA3*. We identified two contrasting combinations of clonal traits, characterizing "*above-ground splitters*" and "*below-ground integrators*", respectively. Clonal traits appeared to play a key role in plant assembly in the studied wet meadows. The interaction of both environmental factors selected for specific combinations of clonal traits, but flooding had a stronger filtering effect than grazing. The hygrophilous community was dominated by "above-ground splitters", while the meso-hygrophilous vegetation was dominated by "below-ground integrators". In the mesophilous community, clonal composition was the most diverse and shared clonal traits with the vegetation of both the hygrophilous and meso-hygrophilous communities. Grazing impacts on CGOs and combinations of clonal traits were different in each community and thus depended on the flooding regime. The ecological and adaptive significance of clonal responses to flooding and grazing are further discussed.

Key words

Clonal traits; combined environmental gradients; plant community structure; wet meadow.

Introduction

In wet meadows traditionally submitted to grazing, such as those commonly found on the French Atlantic coast, the structure of the vegetation is under the control of both flooding and grazing. Although often seasonal, these two factors remain unpredictable and temporarily variable, while their localized effects generate small-scale spatial heterogeneity of environmental conditions (Harper 1977, Blom & Voesenek 1996, Crawford 1996, Adler *et al.* 2001). Both factors have complex effects on plant abiotic and biotic environments. Flooding decreases both the quantity and quality of light available for the plants, modifies nutrient availability and inhibits oxygen diffusion, generating anoxic conditions (Blom & Voesenek 1996). Grazing directly applies to the plants through defoliation and trampling. It may also change plant-to-plant interactions by the generation of canopy gaps and modify the abiotic environment through feces and urine hits or soil compaction (Harper 1977, Huntly 1991).

Flooding and grazing influence species richness and composition and are expected to favor tolerant plants *i.e.* plants that are able to grow and reproduce in the altered habitat conditions they generate (Briske 1996, van Eck *et al.* 2004, del-Val & Crawley 2005, Banach *et al.* 2009). The co-occurrence of these factors has previously been shown to lead to singular interacting effects on plant community structure (Chaneton & Facelli 1991, Oesterheld & McNaughton 1991, Insausti *et al.* 1999, Jutila 1999). Strategies of plant tolerance to flooding and grazing have been widely studied, especially regarding anatomical and morphological traits (see for instance Blom & Voesenek 1996, Briske 1996, Blom 1999, Diaz *et al.* 2007). Moreover, recent studies have highlighted that reproductive traits and especially the ability of clonal growth (*i.e.* vegetative production of genetically identical and potentially autonomous offspring called *ramets*, Harper 1977) are determinant in plant response to flooding (Soukupova 1994, Lenssen *et al.* 2004) and grazing (Diaz *et al.* 2007). However, in such studies, clonality is considered as a trait itself (Weiher *et al.* 1999), despite the great variation and diversity of clonal growth forms (see for instance Grace 1993, Klimeš *et al.* 1997). Indeed, the structures involved in clonal growth, the abilities of spatial exploration and resource storage, the rate of clonal multiplication, or the degree of interaction among ramets are of important ecological and evolutionary significance, and vary among species (Grace 1993, van Groenendael *et al.* 1996, Klimeš *et al.* 1997, Klimešovà & Klimeš 2008). The lack of consideration of clonal traits is probably due to their difficult measurement in the field (Weiher *et al.* 1999, Klimešovà & de Bello 2009). Plant databases constitute an efficient surrogate to field-measured traits but often miss detailed information on clonal traits.

Table 1 – Expected plant characteristics involved in flooding and grazing tolerance, and related assumptions on clonal growth organs and clonal trait values.

Mechanism of resistance	Associated characteristics	References	Expected corresponding clonal growth organs	Expected corresponding clonal traits
Flooding				
Stress tolerance (shade, anoxia)	Long floating stems/leaves	[1; 2]	Stolons	High distance of lateral spread
Stress tolerance (shade, anoxia)	Resource storage/reallocation	[3]	Resource storage organs	Long-lived connections
Temporal or spatial avoidance	Water-borne propagules	[2]	Plant fragments	Free dispersal Short-lived connections
Grazing				
Spatial avoidance	Prostrate/decumbent stature	[4; 5]	Rhizomes Running stolons	Below-ground bud bank
Temporal avoidance	Asynchronous development	[4]		Annual ramets
Morphological tolerance	Large number of meristems	[4]		Big bud banks High clonal multiplication rate
Compensatory growth	Resource storage/reallocation	[4]	Resource storage organs	Long-lived connections

Numbers in brackets refer to the following references: [1] Jackson and Drew (1984), [2] Blom and Voesenek (1996), [3] Crawford and Brändle (1996), [4] Briske (1996), [5] Diaz *et al.* (2007).

The free database *CLO-PLA3* (Klimešovà & Klimeš 2008, Klimešovà & de Bello 2009) records species-specific clonal traits. It provides crucial information for document species-specific clonal trait values and gives the opportunity to compare clonal strategies at the community level.

Through the use of the CLO-PLA3 database, we aimed to provide further insights in the studies of plant clonal traits and to determine the relevance of clonal strategies (defined as syndromes of clonal traits) along combined gradients of flooding and grazing. We specifically aimed to answer the following two questions:

1- To which extent does the combination of the grazing and flooding regime influence clonal traits in wet meadows? In particular, we hypothesized that these two environmental factors should favor clonal growth organs and clonal traits that provide plants with the ability to resist to the conditions they generate (see Table 1 for detailed assumptions).

2- Are the effects of flooding and grazing on clonal traits independent or interactive? In this latter case, does one of the two factors present a stronger structuring effect on the expression of clonal traits?

Methods

Study site

This study was conducted on a grazed wet permanent meadow situated in the Marais Poitevin on the French Atlantic coast (46°26'20"N; 1°12'12"W). The climate is a mild Atlantic type. This wet grassland was reclaimed from tidal salt-marshes in the 10^{th} century and has since been grazed by cows and horses. The soil is characterized by a very clayey texture and a markedly hydromorphic character. A topographical gradient consisting of depressions, higher-level flats and intermediate slopes (with a maximum altitudinal range of 70 cm) occurs repeatedly within the grassland. The flats, which are never flooded, present a mesophilous (M) plant community characterized by grasses and sedges such as *Cynosurus cristatus*, *Lolium perenne*, *Elytrigia repens* and *Carex divisa*. At the level of the intermediate slopes, flooding duration and water levels are variable. On average, flooding occurs from a few weeks to three months a year. These slopes have a meso-hygrophilous (MH) plant community and are characterized by residual soil salinity and sub-halophytic species such as *Juncus gerardii*, *Alopecurus bulbosus* and *Parapholis strigosa*. The depressions are flooded from

winter to early spring, with a maximal water depth attaining 30-40 cm. They have a hygrophilous (H) plant community with flood-tolerant species such as *Agrostis stolonifera*, *Glyceria fluitans* and *Eleocharis palustris.* From here onward, the term *community* will be used to designate these three vegetation types occurring along the flooding gradient.

The study was carried out on the grassland common of Magnils-Reignier (250 ha) where an experimental grazing design has been set up since 1995 to investigate the consequences of grazing scenarios on patterns of plant communities (Rossignol *et al.* 2006). Five experimental paddocks corresponding to a stocking rate ranging from 0, 1, 2, 3 to 4 cows.ha^{-1} (*i.e.* from 0 to 1370 kg.ha^{-1}, Ménard *et al.* 2002) were studied (Rossignol *et al.* 2006). Henceforth, these paddocks will be abbreviated as S0, S1, S2, S3 and S4 respectively, with the number corresponding to the stocking rate. The first paddock was a 4 ha exclosure, ungrazed since 1995, and the cow-grazed paddocks were 1 ha-large. The mesophilous, meso-hygrophilous and hygrophilous communities occurred in the exclosure as well as in each grazed paddock, where they were regularly grazed by the cattle (Loucougaray *et al.* 2004, Rossignol *et al.* 2006).

Vegetation sampling and CLO-PLA3 trait monitoring

We carried out this study in July 2002. This time of the year corresponded to the expected biomass peak, when most of the species were represented in the plant communities. However, some of the early annuals had likely completed their phenological cycle and might have been absent in the relevés. In each relevé, we evaluated the relative abundance of each species as the relative percentage cover in squared quadrats of 0.065 m². We carried out 15 relevés in each combination of community × stocking rate, which resulted in 15 × 3 × 5 = 225 relevés.

We recorded 37 species in the relevés, of which 7 were non-clonal. These latter species represented 0.3 % of the vegetation cover on average. According to the community, the number of clonal species ranged from 13 (mesophilous) to 23 (hygrophilous). According to the stocking rate, it ranged from 15 (S0) to 22 (S2). Non-clonal species were removed from the matrix relevés × species abundance (matrix A), as was the percentage of bare soil (which represented on average 5 % of the cover), in order to focus only on the clonal plants. We then recalculated the percentage cover of each species so that the total cover of each relevé was 1 (Pakeman 2004). The corresponding matrix A', which was not weighted by species abundances, was created using species composition expressed in presence/absence.

For each species, clonal traits and clonal growth organs (CGOs) were documented from the free database CLO-PLA3 (Klimešovà & Klimeš 2008, Klimešovà & de Bello 2009).

CGOs can be defined as organs that "bear a vegetative bud-bank and, provide vascular connections between shoots" (Kleyer *et al.* 2008). All species except *Carex divisa* were registered in the CLO-PLA3 database. For this latter species, we attributed the traits and CGOs of the morphologically and phylogenetically close *Carex disticha*. On the basis of our assumptions of flooding and grazing impacts on CGOs and clonal traits (Table 1), we selected six traits related to clonal morphological characteristics and likely to respond either to flooding, grazing or both (Klimešovà & Klimeš 2008). These were (i) the size of the above-ground bud bank and (ii) the below-ground bud bank, (iii) the ramet life span, (iv) the duration of physical integration (connection life span), (v) the clonal multiplication rate and (vi) the lateral spread distance (Table 2). Species recorded in this study corresponded to seven types of CGOs (Table 2).

Table 2 – Characteristics of clonal growth organs and clonal traits monitored in the CLO-PLA3 database. From Klimešovà and Klimeš (2008), Klimešovà *et al.* (2008), Klimešovà & de Bello (2009).

Trait	Attribute	Abbreviation
Clonal Growth Organ (CGO)	Stolon	CGO1
	Short Epigeogenous Rhizome	CGO9
	Long Hypogeogenous Rhizome	CGO10
	Plantlet/Plant fragment	CGO4-5
	Root Splitter/Root with adventitious buds	CGO14-15
Above-ground bud bank	Big	BBAbB
	Small	BBAbS
Below-ground bud bank	Big	BBBelB
	Small	BBBelS
Shoot life span	1 year (annual ramets)	ShLS1
	>1 year (perennial ramets)	ShLS2
Connection life span (duration of physical integration)	1 – 2 years (splitter)	Integ1
	>2 years (integrator)	Integ2
Clonal multiplication rate	≤ 1 ramet/parent ramet/year	CMR1
	2 – 10 ramets/parent ramet/year	CMR2-10
	> 10 ramets/parent ramet/year	CMR10
Distance of lateral spread	< 0.01m/year (short)	Spr1
	0.01 – 0.25 m/year (medium)	Spr1-25
	> 0.25m/year (long)	Spr25
	Dispersable propagules (free dispersal)	SprDisp

Species × clonal trait and species × CGOs matrices

In the CLO-PLA3 database, clonal traits and CGOs are either categorical or semi-quantitative, and their values are recorded as attributes (Klimešovà & Klimeš 2008). Categorical attributes of the traits and CGOs corresponded to columns in the matrix species × clonal traits (matrix B) and in the matrix species × CGOs (matrix C), respectively. In order to take into account the intra-specific variability of a trait, its attributes were calculated through fuzzy coding, the sum of the scores of a trait being 1 (Pakeman *et al.* 2002, Pakeman 2004, de Bello *et al.* 2005). For instance, for a species that can produce stolons (CGO1), epigeogenous (CGO9) and hypogeogenous stems (CGO10), the scores for CGO1, CGO9 and CGO10 were 0.33, while the scores for the other CGOs (CGO4, CGO5, CGO14 and CGO15) were 0.

The matrix relevés × clonal traits (matrix D), which resulted from the multiplication of matrices A and B, held the abundance of trait attributes in each relevé. Similarly, the matrix relevés × CGOs (matrix E) was obtained by the multiplication of matrices A and C. CGOs only refer to the type of clonal growth organs, while clonal traits go further and can be used to describe the clonal strategies more precisely. However, the expression of some clonal traits may be correlated with the type of CGO. We thus analyzed CGOs separately from clonal traits. In order to check that the results did not only depend on species abundances and on the eventual patterns of species dominance, we created unweighted matrices (matrix D' and matrix E') in a similar way, by respectively multiplying matrices B and C with matrix A' (Pakeman *et al.* 2009).

Data analyses

In order to test the effect of flooding and grazing on the abundance of CGOs, we applied linear model ANOVAs with the community and the stocking rate as main factors, and the interaction between the community and the stocking rate. As the matrix E contained data ranging between 0 and 1, we carried out these analyses on arcsine-transformed data (Crawley 2007), whereas data from matrix E' did not need transformation. Tukey's HSD post-hoc tests were applied for post-hoc comparisons.

Multivariate analyses were carried out on matrices D and D'. In order to characterize the syndromes of clonal traits and the effects of the environmental factors (*i.e.* flooding and grazing) on these syndromes, we carried out a Redundancy Analysis (RDA), with the community and the stocking rate as constraining factors. Then, we checked a potential hierarchical effect of these factors on the expression of clonal traits through variance partitioning between both factors. We compared the strength of their respective effect as the

inertia explained by constrained axes in two independent RDAs, with the constraining factors being (i) the community and (ii) the stocking rate, respectively. The significance of the RDAs was tested using ANOVA-like permutation tests (Legendre & Legendre 1998). Then, trajectories of changes in the expression of clonal traits in response to the weakest environmental factor were drawn for each modality of the strongest factor. For this purpose, we proceeded in two steps. We first independently centered the samples per modality of the strongest factor, by the mean of a within-class Principal Component Analysis (PCA). We then carried out a between-class PCA (Dolédec & Chessel 1991), with each modality of the weakest factor corresponding to one class.

We carried out univariate and multivariate analyses with the R software (R Development Core Team 2008). RDAs were carried out with the VEGAN package (Oksanen *et al.* 2008) and Correspondence Analyses (CAs), with the ADE4 package (Chessel *et al.* 2004).

Results

For CGOs as well as for clonal traits, analyses carried out on weighted data and on unweighted data resulted in similar patterns.

Impact of flooding and grazing on CGOs

The relative abundances of stolons (CGO1), short epigeogenous rhizomes (CGO9) and long hypogeogenous rhizomes (CGO10) were significantly impacted by the community, the stocking rate and their interaction (Table 3). Regarding weighted data, the relative abundance of stolons (CGO1) was the highest in the hygrophilous vegetation, intermediate in the mesophilous vegetation and the lowest in the meso-hygrophilous vegetation. The proportion of stolons in both the hygrophilous and the mesophilous communities increased with stocking rates, whereas no effect was detected for the meso-hygrophilous community (Fig. 1A). Short epigeogenous rhizomes (CGO9) were less abundant in the meso-hygrophilous community compared to the two other plant communities. Grazing impacted the abundance of this CGO only in the meso-hygrophilous vegetation, which was significantly lower in the absence of grazing (S0) than in grazed conditions (Fig. 1B). Long hypogeogenous rhizomes (CGO10) dominated the meso-hygrophilous community, whereas the relative abundance of this CGO was intermediate in the mesophilous community and the lowest in the hygrophilous community. Its cover differed significantly according to the stocking rate only for the meso-

hygrophilous community, where a decrease up to 40% was detected between S0 and S2 (Fig. 1C).

Unweighted data resulted in similar conclusions (Table 3), except that the abundance of stolons was the highest in the hygrophilous community, and was not significantly different between either of the other communities. Analyses were not carried out on plantlets, plant fragments, root splitters or roots with adventitious buds (*i.e.* CGOs 4, 5, 14 and 15, respectively), as they were present in very low abundances and they occurred in only a few relevés (Fig. 1D).

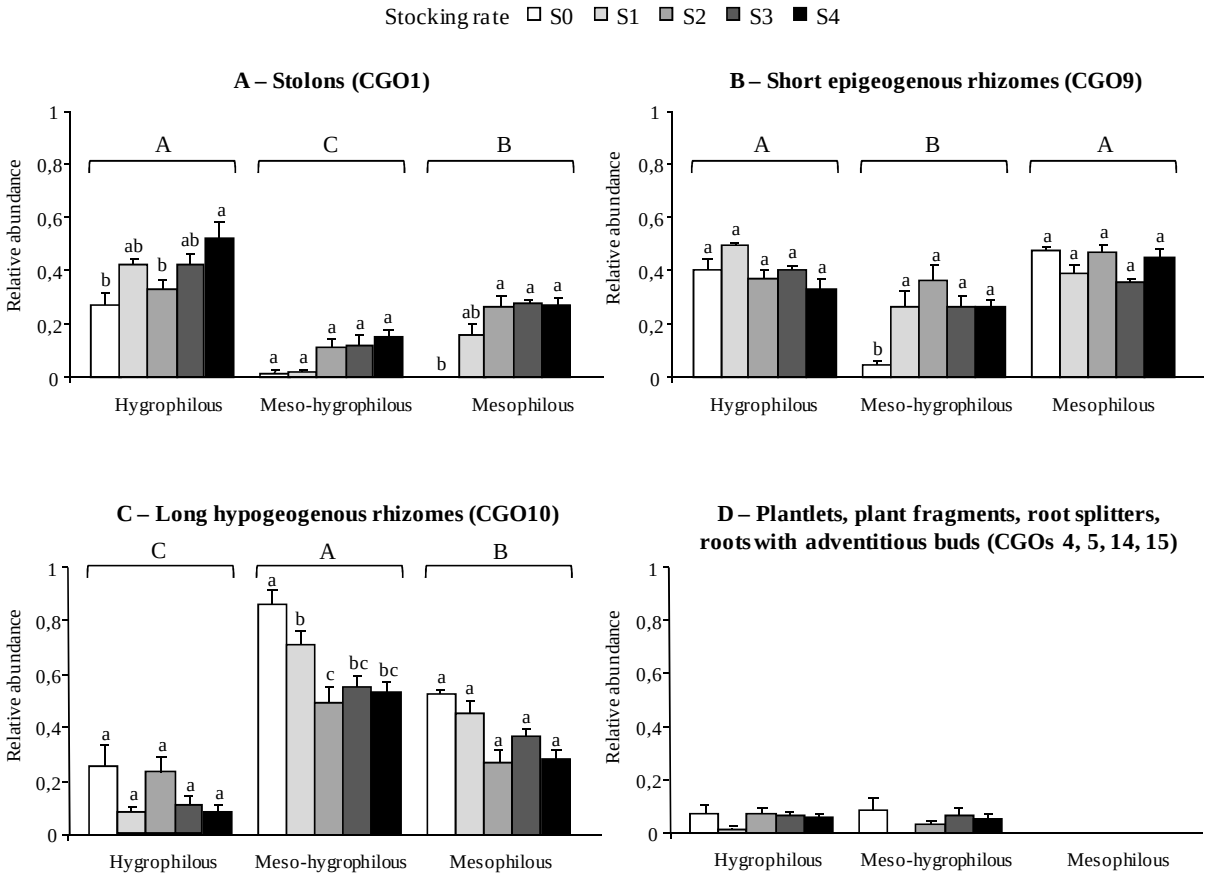

Fig.1 – Relative abundances of clonal growth organs, according to the community and the grazing pressure. (A) Solons (CGO1). (B) Short epigeogenous rhizomes (CGO9). (C) Long hypogeogenous rhizomes (CGO10). (D) Plantlets, plant fragments, root splitters and roots with adventitious buds (*i.e.* CGOs 4, 5, 14 and 15, respectively) represented altogether. Because of very low abundance, these latter ones were not submitted to statistical analyses. Capital letters indicate significant differences between the communities; lower-case letters indicate significant differences among the stocking rates within a community (post-hoc Tukey's HSD tests). Post-hoc comparisons between the community × stocking rate interactions are not shown. S0 –S4: stocking rate.

Table 3 – Results of the linear model ANOVAs on the matrices CGOs × relevés. Figures in the text result from analyses on weighted data (matrix E), those in italics result from analyses on unweighted data (matrix E'). See Table 1 for the significance of the CGOs.

	Community (df=2)		Stocking rate (df=4)		Interaction (df=8)	
	F	*P*	F	*P*	F	*P*
Stolons (CGO1)	103.4	<0.001	17.8	<0.001	2.8	0.005
	63.3	*<0.001*	*10.6*	*<0.001*	*3.17*	*0.002*
Short epigeogenous rhizomes (CGO9)	42.3	<0.001	3.8	0.005	6.9	<0.001
	7.05	*0.001*	*16.1*	*<0.001*	*3.77*	*<0.001*
Long hypogeogenous rhizomes (CGO10)	123.5	<0.001	17.5	<0.001	4.8	<0.001
	84.4	*<0.001*	*3.6*	*0.007*	*6.4*	*<0.001*

Impact of flooding and grazing on clonal traits

RDAs on weighted data showed that the community and the stocking rate taken together explained 41.3 % of the total variance of clonal traits in the vegetation. Both of these environmental factors explained a significant proportion of the variance. However, the community was the strongest factor influencing clonal strategies, as it explained 31.4 % of the total variance, while the stocking rate only explained 10 % (Table 4).

Table 4 – Values of the constrained variance (percentage of the total variance) and results of the ANOVA-like permutation tests for the three RDAs with community, stocking rate or both as constraining factors.

Constraining factor	Total variance	Constrained variance	df	Pseudo-F	*P*-value
Community + stocking rate	0.8105	0.3350 (41.3%)	6	25.6	0.005
Community	0.8105	0.2543 (31.3%)	2	50.8	0.005
Stocking rate	0.8105	0.0081 (10.0%)	4	6.1	0.005

An RDA was carried out on weighted data (matrix D), with the community and stocking rate as environmental constraining factors. Clonal traits were dispersed mainly along the first axis, which was positively correlated to a big above-ground bud bank (BBAbB) and a small below-ground bud bank (BBBelS), short-lived connections (Integ1), perennial ramets (ShLS2) and dispersible propagules (SprDisp; Fig. 2A). These traits were characteristic of hygrophilous relevés (Fig. 2B). On the contrary, this axis was negatively correlated with a small above-ground bud bank (BBAbS) and big below-ground bud bank (BBBelB), long-lived connections (Integ2) and annual ramets (ShLS1; Fig. 2A). This combination of traits

seemed to occur mainly in the meso-hygrophilous vegetation. (Fig. 2B). The second axis explained only 2.2 % of the total variance. Relevés of the mesophilous community were dispersed along the first and second axes. Regarding grazing, the main differences in syndromes of clonal traits occurred between the exclosure (S0), the lightest stocking rate (S1) and the three other stocking rates with no distinction between them (S2 to S4; Fig. 2B).

Similar results were obtained with the unweighted data. The emerging patterns were also very similar to those described previously, except that the first axis carried traits related to shoot life span to a lesser extent.

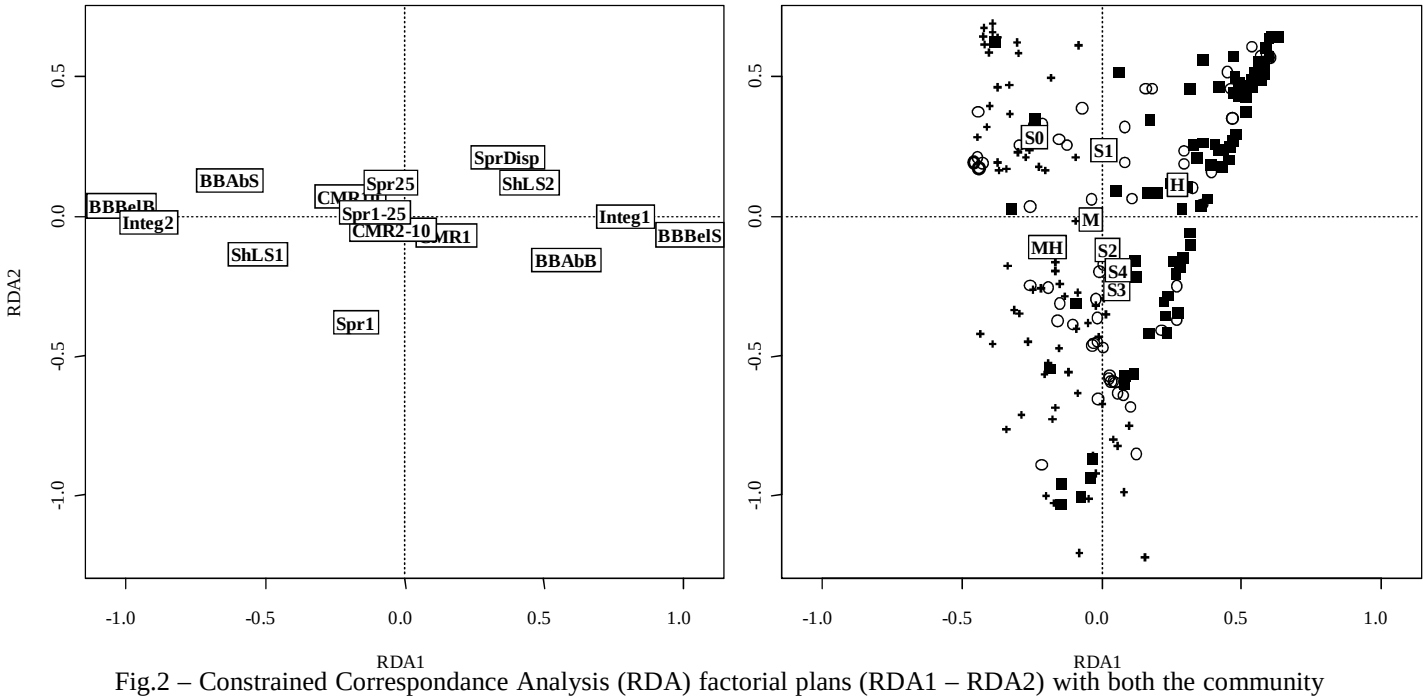

Fig.2 – Constrained Correspondance Analysis (RDA) factorial plans (RDA1 – RDA2) with both the community and the stocking rate as constraining factors. A – Factorial plan of the clonal traits. B – Factorial plan of the relevés and the centroids of environmental factors. See Table 1 for trait abbreviations. Filled squares: hygrophilous, crosses: meso-hygrophilous and open circles: mesophilous relevés. H: hygrophilous, MH: meso-hygrophilous and M: mesophilous communities. S0 – S4: stocking rate.

As community appeared to influence the expression of clonal traits more strongly than the stocking rate, we performed the within–between PCA to analyze clonal trait responses to grazing for each community. The two first axes represented respectively 59.0 % and 20.3 % of the total inertia. Except for the positive correlation with dispersible propagules (SprDisp) and in addition to a negative correlation with long distance spreading (Spr25) and high multiplication rate (CMR10), the first axis was correlated to the same traits as in the RDA (Figs. 2A and 3A). The second axis only concerned the distance of lateral spread, as it was

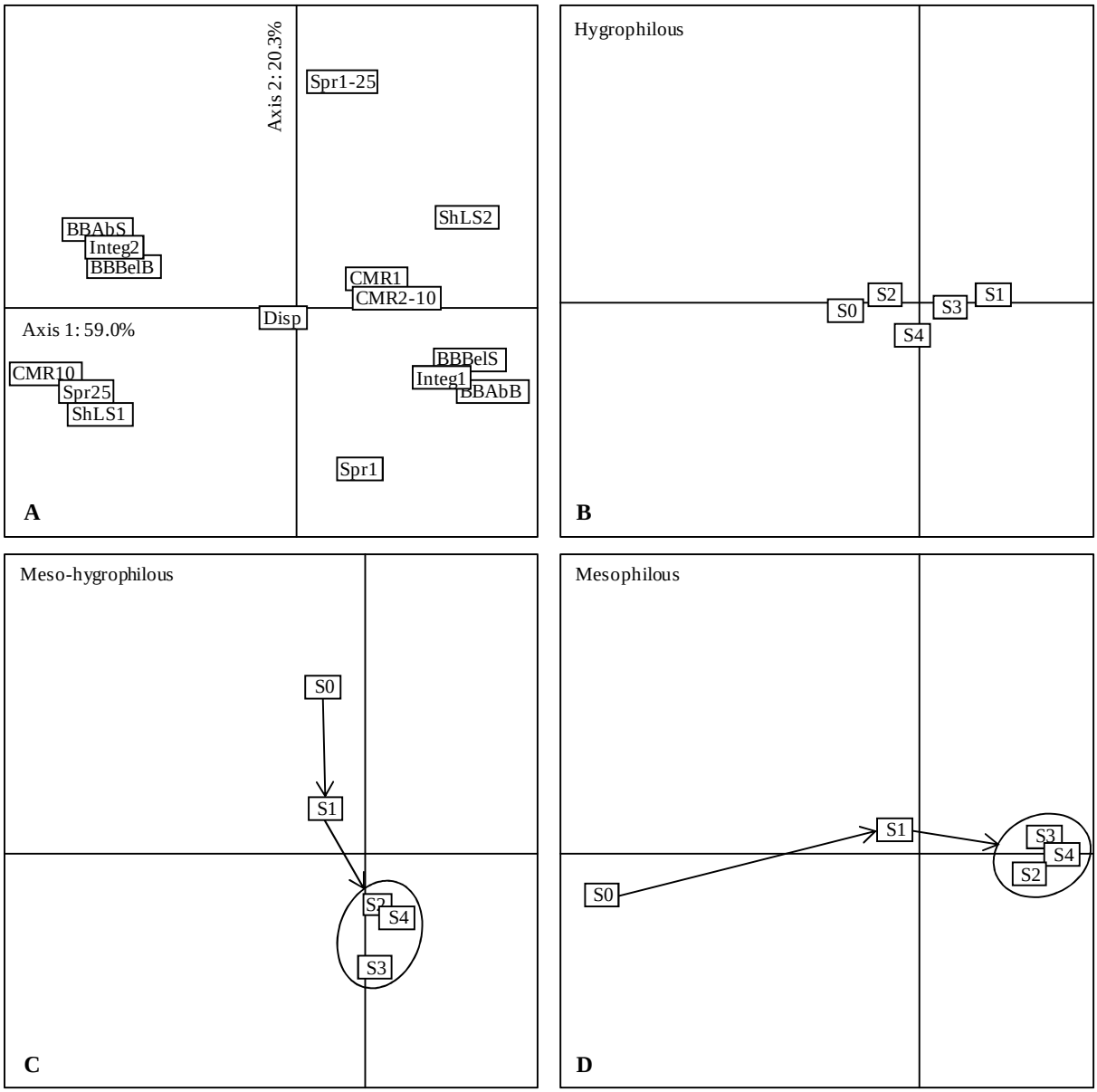

Fig.3 – Within–Between Principal Component Analysis factorial plans (F1 – F2). (A) Factorial plan of the clonal traits. (B –D) Factorial plan of the centroids of relevés grouped according to the stocking rate (hygrophilous, meso-hygrophilous and mesophilous communities respectively). See Table 1 for trait abbreviations. S0 – S4: stocking rate.

positively correlated with moderate distance spreading (Spr1-25) and negatively with short distance spreading (Spr1; Fig. 3A). Grazing impact on the clonal traits depended on the communities. The stocking rate did not affect clonal traits in the hygrophilous community (Fig. 3B). Clonal response to increasing stocking rates occurred along the second axis in the meso-hygrophilous vegetation (Fig. 3C), while this was observed along the first axis for the mesophilous vegetation (Fig. 3D). For both of these communities, clonal response to grazing occurred mainly between the exclosure (S0), the lowest (S1) and the three highest stocking rates altogether (S2 – S4). The only slight differences between the within–between PCA on weighted and unweighted data were the same as those detected for the RDA (see above).

Discussion

Clonal strategies

In our study, 30 out of the 37 species recorded (*i.e.* 81 %) were clonal and represented 97.7 % of the vegetation cover indicating the particular importance of clonal growth in plant community structure. Stem-derived CGOs, *i.e.* stolons (CGO1), short epigeogenous rhizomes (CGO9) and long hypogeogenous rhizomes (CGO10) dominated the vegetation regardless of the community and the stocking rate. These results are in accordance with previous results showing the importance of stem-derived CGOs in plant communities (van Groenendael *et al.* 1996, Klimeš *et al.* 1997, Klimešovà & Klimeš 2008). Syndromes of clonal traits emerged, with correlations between the size and location (below *vs.* above-ground) of the bud banks and the lifespan of ramets and connections. In particular, we detected two contrasting combinations of traits: the first combination, characterized by a large above-ground bud bank, perennial ramets and short-lived connections, could be referred to as "*above-ground splitters*" and the second one, characterized by a large below-ground bud bank, annual ramets and long-lived connections, corresponded to "*below-ground integrators*".

A hierarchical impact of flooding over grazing on clonal strategies

Flooding (*i.e.* community) and grazing (*i.e.* stocking rate) did influence the composition of clonal traits in the vegetation. However, the effects of both of these environmental factors on clonal traits were not independent and were rather interactive, as previously demonstrated for non-clonal traits (Chaneton & Facelli 1991, Oesterheld & McNaughton 1991, Insausti *et al.* 1999, Jutila 1999). Flooding had a more determinant impact than grazing, as shown by variance partitioning with the RDAs. Flooding impacted both the type and heterogeneity of

the combination of clonal traits selected. As expected according to the hypothesis of flooding tolerance (Table 1), the hygrophilous vegetation corresponded to a homogeneous syndrome of traits characteristics of "above-ground splitters" and the dominance of CGOs of above-ground origin (*i.e.* stolons and short epigeogenous rhizomes). These characteristics may present several advantages for the survival of the clone during the stressful growing conditions while flooded. Stolons are often photosynthetic and able to float, and have been suggested as organs of light foraging (Dong & de Kroon 1994, Dong & Pierdominici 1995). They may therefore contribute to light harvesting during the flooding event, while the production of long internodes may decrease the effects of mechanical problems linked to the elevation of the water level (Klimeš *et al.* 1997, Puijalon *et al.* 2008). Stolons are also able to store resources in parenchymatous cells that may enable the genet to cope with the reduced photosynthesis (Crawford & Brändle 1996) and to enhance the survival of plant fragments after disintegration (Stuefer & Huber 1999). Moreover, the production of dispersed fragments is efficient to cope with unpredictable flooding and is particularly important for long-distance spreading while the soil is flooded (Grace 1993, Barrat-Segretain *et al.* 1998). Short-lived connections are little costly (Grace 1993, Santamaria 2002) and may be particularly advantageous in the hygrophilous vegetation, for which growth is limited neither by water nor nutrients, making the mother-to-daughter ramet support unnecessary (Klimeš *et al.* 1997). The above-ground strategy can also promote spatial colonization after the flooding event as above-ground connections are easily able to anchor in the soil through adventitious roots, which are characteristic of flood-tolerant species (Blom & Voesenek 1996).

By contrast, the combination of clonal traits in the mesophilous community appeared to be less homogeneous than in the hygrophilous community. The vegetation was characterized by the co-dominance of the three major CGOs and the emergence of no particular syndrome of clonal traits. This large clonal diversity may result from the absence of flood-related stress, which has already been shown to result in higher specific richness (Chaneton & Facelli 1991, Mesléard *et al.* 1999).

The meso-hygrophilous community did not present intermediate clonal strategies between both extremities of the flooding gradient. On the contrary, it was characterized by the combination of large below-ground bud banks, and long and rather long-lived rhizomes, which we qualified as the syndrome of "below-ground integrators". The slopes where the meso-hygrophilous community occurs present relatively important soil conductivity (Bonis *et al.* 2005). Clonal integration through rhizomes has been suggested as an important trait of plant species in salt marshes (Pennings & Callaway 2000), since it enables the ramets to share

water and to buffer saline conditions. Our results thus suggested that more than flooding, residual salinity in the soil might explain this syndrome of traits for the meso-hygrophilous community.

A community-specific response of clonal strategies to grazing

Although significant, the influence of the stocking rate on the expression of clonal traits was weaker than the community effect. This influence seemed rather qualitative, as there was no difference (in terms of clonal traits) between the three highest stocking rates (Fig. 2A). Clonal response to grazing alone was not predictable and depended on the community, *i.e.* of the position of the vegetation along the flooding gradient. These results are in accordance with previous studies demonstrating that despite a certain consistency, plant responses to grazing were only predictable at a local scale, as it was strongly influenced by other environmental factors such as site productivity (Pakeman 2004), climate (de Bello *et al.* 2005, Diaz *et al.* 2007) or grazing history (Diaz *et al.* 2007). We did not detect a significant change in the plant traits in the hygrophilous community due to the strongest effect of stressful conditions. On the contrary, clonal traits differed in response to grazing in the two other communities. As previously shown when considering all of the communities without distinction, these differences only occurred between the exclosure (S0), the lowest stocking rate (S1) and the three other rates (S2 – S4; Fig. 3). Regardless of the community, the response of clonal traits to grazing did not match our hypotheses (Table 1).

In the meso-hygrophilous vegetation, grazing mainly decreased the distance of lateral spread. This was linked with a change in the proportions of CGOs with a decrease in the long hypogeogenous rhizomes (CGO10) and an increase in the short epigeogenous rhizomes (CGO9). These results are in accordance with the work of Tamm *et al.* (2002), who demonstrated that long distance spreading is characteristic of abandoned grasslands and that clonal mobility tends to be lower in open grasslands. Grazing or mowing have been shown to limit the investment in lateral spread (Moen *et al.* 1999, Sammul *et al.* 2004, Gross *et al.* 2007). A high distance spreading should be particularly advantageous in competitive situations such as in the ungrazed situations dominated by dense paucispecific stands (Grime 1977). Moreover, the maintenance of long connections may represent high costs for the genet (van Groenendael *et al.* 1996). This may be particularly true in disturbed habitats where tissue loss frequently occurs, and may limit or divert biomass production.

The impacts of grazing in the mesophilous community were rather inconsistent with assumptions emerging from the grazing resistance hypothesis (Table 1). Contrary to our

expectations of a dominance of traits characteristic of the "below-ground integrators", grazing significantly increased the relative abundance of stolons and traits corresponding to the "above-ground splitters". Stoloniferous growth forms have already been shown to be favored by grazing (Diaz *et al.* 2007). Resprouting (*i.e.* the production of new ramets after defoliation) depends on the availability and activity of meristems (Briske 1996). Buds located close to the ground are generally left ungrazed. The regrowth after defoliation may also be sustained by carbohydrates stored in above-ground clonal organs and tiller bases (Iwasa & Kubo 1997, Stuefer & Huber 1999, Suzuki & Stuefer 1999, Klimeš & Klimešovà 2002). In addition, the above-ground strategy is, among other properties, characterized by short-lived connections, which implicitly means that young ramets quickly become independent of their mother support. This property could be advantageous in grazed pastures where trampling is likely to damage connections. The ability of stolons to quickly produce adventitious roots may promote their anchoring when trampling pins the above-ground tissues to the ground. By contrast, this trait may be disadvantageous in ungrazed areas, as the high canopy may prevent stolons from growing close to the soil surface and to root successfully.

Conclusions

Following previous studies on plant responses to grazing (see for instance Pakeman 2004, de Bello *et al.* 2005, Diaz *et al.* 2007), our results provide further evidence that the impacts of grazing on plant functional traits strongly depend on the pool of traits available. Plant traits appear to be at first filtered by environmental factors constraining abiotic conditions (*e.g.* climate, site productivity, flooding). Regarding clonal traits, it even emerged from our results that such a hierarchical effect can occur at a very local scale (*i.e.* within a single paddock). Further studies on non-clonal plant functional traits at such a small scale could test the consistency of this result.

The CLO-PLA3 database provides crucial information on clonal traits needed for the analyses of clonal patterns at the community level. It is all the more interesting as clonal traits remain hardly measured *in situ*. Although the CLO-PLA3 database deals with averaged traits collected from diverse sources, the present study is an example that it can be applied to site-specific environmental gradients. The combination of databases and field observations leads to emerging hypotheses that can then be tested through experiments, in particular dealing with the relative importance of inter *versus* intra-specific variability.

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Literature cited

Adler PB, Raff DA & Laurenroth WK (2001) The effect of grazing on the spatial heterogeneity of vegetation. *Oecologia*, 128: 465-479.

Banach K, Banach AM, Lamers LPM, de Kroon H, Bennicelli RP, Smits AJM & Visser EJW (2009) Differences in flooding tolerance between species from two wetland habitats with contrasting hydrology: implications for vegetation development in future floodwater retention areas. *Annals of Botany*, 103: 341-351.

Barrat-Segretain MH, Bornette G & Hering-Vilas-Bôas A (1998) Comparative abilities of vegetative regeneration among aquatic plants growing in disturbed habitats. *Aquatic Botany*, 60: 201-211.

Blom CWPM (1999) Adaptations to flooding stress: from plant community to molecule. *Plant Biology*, 1: 261-273.

Blom CWPM & Voesenek LACJ (1996) Flooding: the survival strategies of plants. *Trends in Ecology and Evolution*, 11: 290-295.

Bonis A, Bouzillé JB, Amiaud B & Loucougaray G (2005) Plant community patterns in old embanked grasslands and the survival of halophytic flora. *Flora*, 200: 74-87.

Briske DD (1996) Strategies of plant survival in grazed systems: a functional interpretation. In: Hodgson J & Illius AW (eds) *The Ecology and management of grazing systems*. CAB International, Wallingford, pp 33-67.

Chaneton EJ & Facelli JM (1991) Disturbance effects on plant community diversity: spatial scales and dominance hierarchies. *Plant Ecology*, 93: 143-155.

Chessel D, Dufour AB & Thioulouse J (2004) The ade4 package – I – One-table methods. *R News*, 4: 5-10.

Crawford RMM (1996) Whole plant adaptations to fluctuating water tables. *Folia Geobotanica and Phytotaxonomica*, 31: 7-24.

Crawford RMM & Brändle R (1996) Oxygen deprivation stress in a changing environment. *Journal of Experimental Botany*, 47: 145-159.

Crawley MJ (2007) *The R book*. John Wiley & Sons, Chichester.

de Bello F, Leps J & Sebastià MT (2005) Predictive value of plant traits to grazing along a climatic gradient in the Mediterranean. *Journal of Applied Ecology*, 42: 824-833.

del-Val E & Crawley MJ (2005) Are grazing increaser species better tolerators than decreasers? An experimental assessment of defoliation tolerance in eight British grassland species. *Journal of Ecology*, 93: 1005-1016.

Diaz S, Lavorel S, McIntyre S, Falczuk V, Casanoves F, Michunas DG, Skarpe C, Rusch G, Sternberg M, Noy-Meir I, Landsberg J, Zhang W, Clark H & Campbell BD (2007) Plant trait responses to grazing – a global synthesis. *Global Change Biology*, 13: 313-341.

Dolédec S & Chessel D (1991) Recent development in linear ordination methods for environmental sciences. *Advances in Ecology*, 1: 133-155.

Dong M & de Kroon H (1994) Plasticity in morphology and biomass allocation in *Cynodon dactylon*, a grass species forming stolons and rhizomes. *Oikos*, 70: 99-106.

Dong M & Pierdominici MG (1995) Morphology and growth of stolons and rhizomes in three clonal grasses, as affected by different light supply. *Vegetatio*, 116: 25-32.

Grace J (1993) The adaptive significance of clonal reproduction in angiosperms: an aquatic perspective. *Aquatic Botany*, 44: 159-180.

Grime JP (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist*, 111: 1169-1194.

Gross N, Suding K & Lavorel S (2007) Leaf dry matter content and lateral spread predict response to land use change for six subalpine grassland species. *Journal of Vegetation Science*, 18: 289-300.

Harper JL (1977) *Population Biology of Plants*. Academic Press, London.

Huntly N (1991) Herbivores and the dynamic of communities and ecosystems. *Annual Review of Ecology and Systematics*, 22: 477-503.

Iwasa Y & Kubo T (1997) Optimal size of storage for recovery after unpredictable disturbances. *Evolutionary Ecology*, 11: 41-65.

Insausti P, Chaneton EJ & Soriano A (1999) Flooding reverted grazing effects on plant community structure in mesocosms of lowland grassland. *Oikos*, 84: 266-276.

Jackson MB & Drew MC (1984) Effect of flooding on herbaceous plants. In: Kozlowski TT (ed) *Flooding and plant growth.* Academic Press, London, pp 47-128.

Jutila H (1999) Effect of grazing on the vegetation of shore meadows along the Bothnian Sea, Finland. *Plant Ecology*, 140: 77–88.

Kleyer M, Bekker RM, Knevel IC, Bakker JP, Thompson K, Sonnenschein M, Poschlod P, van Groenendael JM, Klimeš L, Klimešová J, Klotz S, Rusch GM, Hermy M, Adriaens D, Boedeltje G, Bossuyt B, Dannemann A, Endels P, Götzenberger L, Hodgson JG, Jackel AK, Kühn I, Kunzmann D, Ozinga WA, Römermann C, Stadler M, Schlegelmilch J, Steendam HJ, Tackenberg O, Wilmann B, Cornelissen JHC, Eriksson O, Garnier E & Peco B (2008) The LEDA Traitbase: A database of life-history traits of Northwest European flora. *Journal of Ecology*, 96: 1266-1274.

Klimeš L & Klimešovà J (2002) The effects of mowing and fertilization on carbohydrate reserves and regrowth of grasses: do they promote plant coexistence in species-rich meadows? *Evolutionary Ecology*, 15: 363-382.

Klimeš L, Klimešovà J, Hendricks R & van Groenendael J (1997) Clonal plant architecture: a comparative analysis of form and function. In: de Kroon H & van Groenendael J (eds) *The ecology and evolution of clonal plants*. Backhuys Publishers, Leiden, pp 1-29.

Klimešovà J & de Bello F (2009) CLO-PLA: the database of clonal and bud bank traits of Central European flora. *Journal of Vegetation Science*, 20: 511-516.

Klimešovà J & Klimeš L (2008) Clonal growth diversity and bud banks of plants in the Czech flora: an evaluation using the CLO-PLA3 database. *Preslia*, 80: 255-275.

Klimešovà J, Latzel V, de Bello F & van Groenendael JM (2008) Plant functional traits in studies of vegetation changes in response to grazing and mowing: towards a use of more specific traits. *Preslia*, 80: 245-253.

Legendre P & Legendre L (1998). *Numerical Ecology*. 2nd English ed. Elsevier, Amsterdam.

Lenssen JPM, van Kleunen M, Fischer M & de Kroon H (2004). Local adaptation of clonal plant *Ranunculus reptans* to flooding along a small-scale gradient. *Journal of Ecology*, 92: 696-706.

Loucougaray G, Bonis A & Bouzillé JB (2004) Effects of grazing by horses and/or cattle on the diversity of coastal grasslands in western France. *Biological Conservation*, 116: 59-71.

Ménard C, Ducan P, Fleurance G, George JY & Lila M (2002) Comparative foraging and nutrition in horses and cattle in Europe wetlands. *Journal of Applied Ecology*, 38: 120-133.

Mesléard F, Lepart J, Grillas P & Mauchamp A (1999) Effects of seasonal flooding and grazing on the vegetation of former ricefields in the Rhône delta (Southern France). *Plant Ecology*, 145: 101-114.

Moen J, Ingvarsson PK & Walton DWH (1999) Estimates of structural complexity in clonal plant morphology: comparisons of grazed and ungrazed *Acaena magellanica* rhizomes. *Canadian Journal of Botany*, 77: 869-876.

Oesterheld M & McNaughton SJ (1991) Interactive effect of flooding and grazing on the growth of Serengeti grasses. *Oecologia*, 88: 153-156.

Oksanen J, Kindt R, Legendre P, O'Hara B, Simpson GL, Solymos P, Stevens MHH & Wagner H (2008) Vegan: Community Ecology Package. R package version 1.15-1. http://cran.r-project.org/, http://vegan.r-forge.r-project.org.

Pakeman RJ (2004) Consistency of plant species and trait responses to grazing along a productivity gradient: a multi-site analysis. *Journal of Ecology*, 92: 893-905.

Pakeman RJ, Lepš J, Kleyer M, Lavorel S, Garnier E & the VISTA consortium (2009) Relative climatic, edaphic and management controls of plant functional trait signatures. *Journal of Vegetation Science*, 20: 148–159.

Pakeman RJ, Pywell RF & Wells TCE (2002) Species spread and persistence: implications for experimental design and habitat re-creation. *Applied Vegetation Science*, 5: 75-86.

Pennings S & Callaway R (2000) The advantages of clonal integration under different ecological conditions: a community-wide test. *Ecology*, 81: 709-716.

Puijalon S, Bouma TJ, van Groenendael J & Bornette G (2008) Clonal plasticity of aquatic plant species submitted to mechanical stress: escape versus resistance strategy. *Annals of Botany*, 102: 989-996.

R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Rossignol N, Bonis A & Bouzillé JB (2006) Relationships between grazing pattern and vegetation structure on the spatial variations of net N mineralisation in a wet grassland. *Applied Soil Ecology*, 31: 62-72.

Sammul M, Kull K, Niitla T & Möls T (2004) A comparison of plant communities on the basis of their clonal growth patterns. *Evolutionary Ecology*, 18: 443-467.

Santamaria L (2002) Why are most aquatic plants widely distributed? Dispersal, clonal growth and small-scale heterogeneity in a stressful environment. *Acta Oecologica*, 23: 137-154.

Soukupova L (1994) Allocation plasticity and modular structure in clonal graminoids in response to waterlogging. *Folia Geobotanica and Phytotaxonomica*, 29: 227-236.

Stuefer JF & Huber H (1999) The role of stolon internodes for ramet survival after clone fragmentation in *Potentilla anserina*. *Ecology Letters*, 2: 135-139.

Suzuki JI & Stuefer J (1999) On the ecological and evolutionary significance of storage in clonal plants. *Plant Species Biology*, 14: 11-17.

Tamm A, Kull K & Sammul M (2002) Classifying clonal growth forms based on vegetative mobility and ramet longevity: a whole community analysis. *Evolutionary Ecology*, 15: 383-401.

van Eck WHJM, van de Steeg HM, Blom CWPM & de Kroon H (2004) Is tolerance to summer flooding correlated with distribution patterns in river floodplains? A comparative study of 20 terrestrial grassland species. *Oikos*, 107: 393-405.

van Groenendael JM, Klimeš L, Klimešovà J & Hendricks R (1996) Comparative ecology of clonal plants. *Philosophical Transactions of the Royal Society London*, 351: 1331-1339.

Weiher E, van der Werf A, Thompson K, Roderick M, Garnier E & Eriksson O (1999) Challenging Theophrastus: a common core list of plant traits for functional ecology. *Journal of Vegetation Science*, 10: 609-620.

Article 2 – Spatial patterns in defoliation and the expression of clonal traits in grazed meadows

Marie-Lise Benot, Anne Bonis, Nicolas Rossignol & Cendrine Mony

UMR CNRS 6553, ECOBIO, Université de Rennes 1, Campus de Beaulieu, 35042 Rennes Cedex, France

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Abstract

Grazing can generate spatial patchiness at different scales, from very local (< 1 m) to large scales (several 100 m). However grazing-induced patchiness has mainly been studied regarding vegetation composition or soil properties but much less attention has been paid on spatial patterns of direct grazing effects on plants (defoliation). In meadows, the vegetation is dominated by clonal plants, composed of potentially independent descendants (ramets) linked together by connections. Clonality is associated with singular properties, which express differently according to environmental conditions, notably on environmental patchiness. Fine-grained patchiness of defoliation, perceptible at the scale of the clonal fragment, is likely to favor lateral spreading and resource sharing, enabling intact ramets to support damaged ones. Under coarse-grained defoliation, perceived as homogeneous by the clonal plant, resource storage and high multiplication rate are more likely to allow an efficient regrowth.

This study aimed to characterize the patchiness of grazing-induced defoliation and to evaluate its impacts on clonal growth forms. In that purpose, patchiness of vegetation height and grazing impacts on plants were monitored along a cattle-grazing gradient. Species were identified and their clonal traits documented in the free database CLO-PLA3.

Our results showed fine-grained patchiness of the vegetation, independently from the grazing regime. These spatial patterns were thus likely due to intrinsic properties of the vegetation or small scale patterns of abiotic conditions. Moderate grazing tended to increase fine-grained patchiness, while it influenced mainly coarse-grained patchiness. By contrast, intense grazing tended to homogenize vegetation cover. Because of its light impact on fine-grained patchiness, grazing effects were probably perceived as homogeneous by clonal fragments. Grazing favored combinations of clonal traits, which are expected to minimize the costs of clonal growth. On the contrary, lateral spread was favored in ungrazed conditions, probably because it enhanced competitive ability.

Key words

Cattle grazing; clonal traits; defoliation; scale; spatio-temporal patchiness; vegetation height.

Introduction

Grazing is a complex biotic factor, which strongly influences the composition and structure of vegetation (Huntly 1991). In particular, grazing may affect vegetation by generating spatial heterogeneity (patchiness) at different scales, from very local scales, less than 1 m-large to large patterns, several 100 m-large (Rietkerk *et al.* 2000, Adler *et al.* 2001, Augustine 2003, see also Olofsson *et al.* 2008 for the particular case of rabbit grazing). Grazing-induced patchiness is often considered in terms of species composition (Bakker *et al.* 1983, Posse *et al.* 2000, Augustine 2003, Loucougaray *et al.* 2004, Collins & Smith 2006, Oom *et al.* 2008), light availability (Bakker *et al.* 2003, Veen *et al.* 2008) or soil properties (Posse *et al.* 2000, Augustine & Frank 2001, Anderson *et al.* 2004, Zhou *et al.* 2008). Grazing-induced defoliation, which primarily consists of discrete events at the bite scale, could also be heterogeneous at several scales (Schwinning & Parsons 1999, WallisDeVries *et al.* 1999, see also Weber *et al.* 1998). Yet, the spatial pattern of direct impacts of grazing on plants through defoliation has much less been considered (Oom *et al.* 2004, Olofsson *et al.* 2008).

Plant meadow communities are mainly composed of clonal species (Klimeš *et al.* 1997). A clonal fragment consists in potentially independent propagules (the *ramets*) linked together by plagiotropic stem-derived connections. Clonal growth provides plants with singular properties. First, clonality enables clonal fragments to spread horizontally (Hutchings & Mogie 1990) and governs the spatial distribution of ramets through clonal architecture (Bell & Tomlinson 1980, Lovett-Doust 1981, Cain 1994, Wolfer *et al.* 2006). Clonal architectures can be ranged along a gradient from phalanx to guerrilla growth forms (*sensu* Lovett-Doust 1981). Phalanx growth forms are characterized by short and highly branched connections, leading to a compact aggregation of ramets. By contrast, guerrilla growth forms are composed of few but long connections resulting in a dispersed network of ramets. Secondly, clonal growth organs may be involved and even specialized in resource storage (Suzuki & Stuefer 1999). While resource storage mainly occurs in below-ground clonal organs, such as rhizomes, bulbs or tubers (Dong & de Kroon 1994, Dong & Pierdominici 1995, Suzuki & Stuefer 1999), above-ground clonal organs can also fulfill this function (Stuefer & Huber 1999, Suzuki & Stuefer 1999). Clonal integration can also allow substance sharing among inter-connected ramets through the connections (physiological integration, Pitelka & Ashmun 1985, Hutchings & Bradbury 1986, Kelly 1995). While intensive physiological integration concerns only a few ramets and centimeters, extensively integrated connection networks enable the translocation of resources from a few decimeters to meters (D'Hertefeld & Jónsdóttir 1999, D'Hertefeld & Falkengren-Grerup 2002).

The expression of clonal properties is likely to vary according to environmental features. In particular, some properties have been suggested to enable clonal plants to explore and efficiently exploit heterogeneous environments (de Kroon & Schieving 1990, Jónsdóttir & Watson 1997, Hutchings 1999). Guerrilla growth forms may be advantageous in heterogeneous conditions, where long distance lateral spread could enable the clonal fragment to escape from unfavorable conditions (de Kroon & Schieving 1990, Macek & Lepš 2003, Puijalon *et al.* 2008). Under heterogeneous defoliation, ramets may be differentially impacted according to their spatial distribution and loosely aggregated ramet networks could spread the risk of defoliation among ramets. Furthermore, clonal integration has been suggested to enable the clonal fragment to buffer heterogeneous damages by allowing undefoliated ramets to support damaged ones (Hartnett 1989, Jónsdóttir & Callaghan 1989, Herms & Mattson 1992). Connections can also allow the movement of signaling or defense compounds from defoliated ramets to undefoliated ones. The resulting induced systemic resistance, corresponding to a decrease palatability of intact ramets, is likely to prevent them from being damaged (Gómez & Stuefer 2006). However, all of these properties may fail to enable a clonal fragment to cope with homogeneous defoliation, under which other clonal properties are expected to be advantageous. After losses of biomass, the activation of dormant buds allows for vegetative regeneration (*i.e.* resprouting, Belligham & Sparrow 2000). In clonal plant, vegetative regeneration can lead to the production of new ramets, depending on the presence of a vegetative bud bank protected from damage (Klimešová & Klimeš 2003, 2007). Moreover, the presence and the mobilization of stored resources readily after defoliation has been shown to support compensatory growth as well as resprouting (Richards 1993, Iwasa & Kubo 1997, Bell & Ojeda 1999, Lattanzi *et al.* 2004)

The relative involvement of such clonal properties depends on way the clonal fragment perceives its environment. In particular, clonal properties expected to favor responses to environmental patchiness would only be efficient if its scale matches the scale of the clonal plant (Stuefer 1996, Wijesinghe & Hutchings 1997, 1999). When grown under experimental conditions of heterogeneous soil nutrient supply, clonal fragments of *Glechoma hederacea* best perceive patchiness for 0.25×0.25 m patches (Wijesinghe & Hutchings 1997). For half-size and smaller patches, clonal fragments fail to respond to environmental patchiness. Greater patch size than the range of the whole clonal fragment (coarse-grained patchiness) may also be perceived as homogeneous (Stuefer 1996).

Whereas some extremely large clonal fragments have been registered, reaching several hundred square meters and even hectares (McLelann *et al.* 1997, Jónsdóttir *et al.* 2000), these

are exceptions. In general, clonal spread, as well as physiological integration, occurs on some decimeters to meters (D'Hertefeld & Jónsdóttir 1999, D'Hertefeld & Falkengren-Grerup 2002, Kun & Oborny 2003). Consequently, the range of patchiness perceptible by a clonal fragment (fine-grained patchiness) is limited, roughly comprised between ten centimeters and a few meters.

In natural conditions, little is known about the scale of patchiness and more particularly, whether it can be perceived by clonal plants (Fischer & van Kleunen 2002). The objective of this study was to analyze the interaction of environmental patchiness and clonal properties. It was divided into two steps. (1) The first aim was to characterize fine-scale spatio-temporal patterns of defoliation induced by cattle in grazed meadows and to determine whether cattle grazing may generate patchiness at the plant scale. (2) The second aim was to link these spatio-temporal characteristics with clonal properties, described by the means of clonal traits. In particular, the two following hypotheses were tested:

Coarse-grained patchiness, perceived as homogeneous at the clonal fragment scale (*i.e.* more than one meter) would favor clonal traits related to defoliation tolerance (*e.g.* big below-ground bud bank, high clonal multiplication rate and/or storage organs).

(i) Fine-grained patchiness, perceived as heterogeneous at the clonal fragment scale (*i.e.* between ten centimeters and one meter) would favor clonal traits enabling the clonal fragment to avoid defoliated micro-sites (*e.g.* high distance clonal spreading) or traits involved in the translocation of substances among ramets (*e.g.* long-lived connections).

Table 1 – Spatio-temporal indices. The calculation methods and description of each index are indicated.

Index	Calculation	Description
Indices of patchiness		
Mean height	Mean of the vegetation height in each cell of the grid in June 2008	Mean height of the vegetation at the plot level
Global variance	Variance of the vegetation height between each cell of the grid in June 2008	Degree of spatial variation of the height between cells at the plot level - Values close to 0: similarity between all cells (homogeneity) - High values: great variation between all cells of the plot
Local covariance	Covariance of the vegetation height between each cell and its neighbor cells* in June 2008	Degree of covariation of the height between neighbor cells - Values close to 0: similarity between all cells (homogeneity) - Values differing from 0: great variation between all cells of the plot ~ Positive values: similarity between neighbors (fine-grained patchiness) ~ Negative values: dissimilarity between neighbors (randomness)
Defoliation percentage	Percentage of defoliated cells within a plot in June 2008	Percentage of defoliation at the plot level
Indices of temporal variation		
Δ mean height	Mean height in October 2008 – mean height in April 2008	Temporal variability of the mean height during the grazing season
Δ global variance	Global variance in October 2008 – global variance in April 2008	Temporal variability of the global variance during the grazing season
Δ local covariance	Local covariance in October 2008 – local covariance in April 2008	Temporal variability of the local covariance during the grazing season

* Cells are considered as neighbors when they share one side

Material and methods

Study site

This study was carried out in the experimental design of the Magnils-Reigners in the Marais Poitevin, which is located on French Atlantic Coast (46°26'20"N; 1°12'12"W) (Rossignol *et al.* 2006). Field observations and measurements were done in the mesophilous community of three paddocks, corresponding to three contrasted grazing regimes: no, intermediate and heavy stocking rate. The ungrazed paddock (S0) is a 4 ha exclosure from which grazing has been excluded since 1995. The two other paddocks (S2 and S4), which are 1 ha-large, have been submitted to cow-grazing since 1995 with respective stocking rates of 2 and 4 cows.ha^{-1} (*i.e.* about 685 and 1370 kg.ha^{-1}, Ménard *et al.* 2002). They respectively correspond to moderate (S2) and intensive grazing (S4). Vegetation height and floristic composition were measured in ten plots randomly positioned within each of the three paddocks (30 plots in total).

Field measurements

Spatio-temporal characterization of the height of the vegetation cover

We aimed to characterize spatial patterns of grazing-induced defoliation and their temporal variability. Plots consisted of 1 × 1 m grids divided into 0.1 × 0.1 m cells. A square of polystyrene (0.01 m², 2 g) allowed the measurement of the height of the vegetation cover in each cell, without taking rare shoots or branches into account (Westoby 1998).

These measurements were carried out four times: before the grazing season (April 2008), twice during the expected peak of biomass (June and July 2008) and just after the grazing season (October 2008). In order to provide a more accurate index of disturbance independently from vegetation height, we inspected the vegetation and recorded leaf damage in each cell in June 2008.

For each plot, we calculated indices describing the spatial characteristics of the vegetation height and their temporal variation (spatio-temporal indices): (i) mean vegetation height, (ii) global variance of vegetation height, (iii) local covariance of vegetation height, (iv) percentage of defoliated cells, *i.e.* cells in which leaf damage had been observed and (v-vii) temporal variability of indices i to iii (see Table 1 for the calculation methods and the signification of these indices).

Species sampling and CLO-PLA3 trait monitoring

In June 2008, we evaluated the relative abundance of each species and bare soil as their relative percentage cover in 0.5 × 0.5 m subplots placed at the center of each plot. In order to focus only on plants, we removed the percentage of bare soil and recalculated the percentage cover of each species, so that the total cover of each subplot was 1 (Pakeman 2004).

For each species, clonal traits and clonal growth organs (CGOs) were documented from the free database CLO-PLA3 (Klimešovà & Klimeš 2008, Klimešovà & de Bello 2009). We selected six traits related to clonal morphological characteristics, which were (i) sizes of the above-ground and (ii) the below-ground bud banks, (iii) ramet life span, (iv) duration of physical integration (connection life span), (v) clonal multiplication rate and (vi) distance of lateral spread (Table 2).

Table 2 – Characteristics of clonal growth organs and clonal traits monitored in CLOPLA database. From Klimešovà & Klimeš (2008); Klimešovà *et al.* (2008); Klimešovà & de Bello, 2009.

Trait	Attribute	Abbreviation
Clonal Growth Organ	No CGO	CGO0
(CGO)	Stolon	CGO1
	Short Epigeogenous Rhizome	CGO9
	Long Hypogeogenous Rhizome	CGO10
	Plantlet/Plant fragment	CGO4-5
	Root Splitter/Root with adventitious buds	CGO14-15
Above-ground bud bank	Big	BBAB
	Small	BBAS
Below-ground bud bank	Big	BBBB
	Small	BBBS
Shoot life span	1 year (annual ramets)	ShLS1
	>1 year (perennial ramets)	ShLS2
Connection life span	No connection	Integ0
(duration of physical integration)	1 – 2 years (splitter)	Integ1
	>2 years (integrator)	Integ2
Clonal multiplication rate	No clonal multiplication	CMR0
	≤ 1 ramet/parent ramet/year	CMR1
	2 – 10 ramets/parent ramet/year	CMR2-10
	> 10 ramets/parent ramet/year	CMR10
Distance of lateral spread	No clonal spread	Spr0
	< 0.01m/year (short)	Spr1
	0.01 – 0.25 m/year (medium)	Spr1-25
	> 0.25m/year (long)	Spr25
	Dispersable propagules (free dispersal)	SprDisp

Data analyses

Spatio-temporal characteristics of the vegetation cover

We analyzed the spatial pattern of the vegetation height by the means of correlograms. We used Moran's I as the index of autocorrelation of the dependent variable (vegetation height in a cell), which we plotted against the physical distance between points of measurement (Cliff and Ord 1973, 1981, Sokal and Oden 1978). In order to draw one correlogram per grazing regime and per date, we pooled data of the ten replicates of each grazing regime. Significance of the Moran's I was tested by permutation tests for each correlograms. Significantly positive or negative values of I indicate spatial patchiness (*i.e.* patchy distribution of the vegetation height), while not significant values (*i.e.* close to 0) are characteristic of randomness or homogeneity (Adler *et al.* 2001).

We studied the correlations between the seven spatio-temporal indices of the vegetation cover and their relationship with the grazing regime through Principal Component Analysis (PCA).

Relation between spatio-temporal indices and clonal traits

Clonal traits recorded in CLO-PLA3 database are either nominal or semi-quantitative (Klimešovà & Klimeš 2008, Klimešovà & de Bello 2009; Table 2). We created the matrix species × clonal traits, where each attribute of a clonal trait corresponded to a column. In order to take into account the intra-specific variability of a trait, its attributes were calculated through fuzzy coding, the sum of scores of a trait being 1 (Pakeman *et al.* 2002, Pakeman 2004, de Bello *et al.* 2005). We then created the matrix plot × clonal traits by the multiplication of the matrices plot × species and species × clonal traits.

We used a co-inertia analysis between the matrices plot × clonal traits and plot × indices in order to determine if the characteristics of the vegetation height and the clonal traits were related. The significance of the matching between the two matrices was tested by Monte-Carlo permutation test (999 permutations).

The statistical analyses were carried out with the R software (R Development Core Team, 2007, http://www.R-project.org). PCA and co-inertia analysis were carried out with the ADE4 package (Chessel *et al.* 2004).

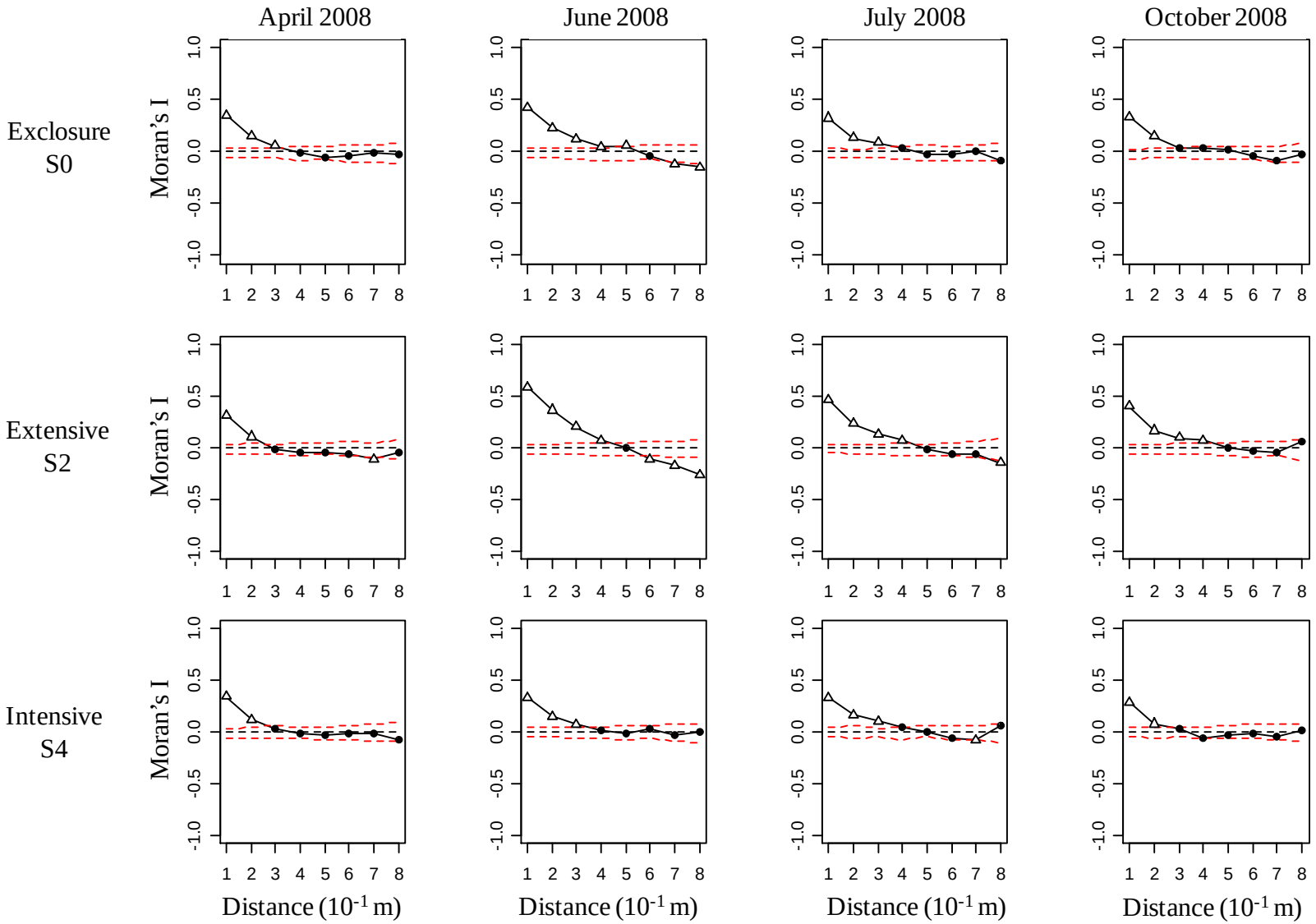

Fig. 1–Autocorrelograms of the height of the vegetation cover for the three grazing regimes and the four dates. Open triangles: significant values of Moran's I ($P < 0.05$), black circles: not significant values of Moran's I ($P > 0.05$).

Results

Spatial pattern of the vegetation height and its temporal variation

Auto-correlograms showed significant positive Moran's I at small scale, which decreased to no correlation as the distance increased (Fig 1). This is an indication of the existence of small patches within the vegetation structure, from 0.2 to 0.5 m-large. The scale of this patchiness depended mainly on the sampling date and, to a lesser extent, on the grazing regime. Patches were the largest in June 2008, reaching 0.5 m-large in the exclosure (S0). During the first months of the grazing season (April – June 2008), patch size decreased with increasing grazing regimes: patches were the largest in the exclosure (S0; 0.3 – 0.5 m) and the smallest under intensive grazing (S4; 0.2 – 0.3 m). This tendency changed during the grazing season, at the end of which patches were 0.2 m-large in the exclosure and under intensive grazing (S0 and S4) and 0.4 m-large under moderate grazing (S2; Fig. 1). At higher scales, non-significant Moran's I values suggested randomness or homogeneity of the vegetation height. In June 2008, negative Moran's I values from 0.6 – 0.7 cm to 1 m represented the gap distance between patches. However, because of the few comparisons at high distances, the reliability of auto-correlograms decreases as the distance increases. These observations should thus be considered with caution.

Patterns of vegetation height in three representative plots are drawn in Fig. 2. The mean height of the vegetation decreased as the grazing regime increased, while the intra-plot variability seemed greater for the intermediate grazing regime (Fig. 2). These observations are confirmed by results of the PCA. The first and second axes of the PCA explained 54.7 % and 25.6 % of the total inertia respectively (Fig. 3). The first axis was positively correlated with the percentage of defoliated cells within a plot, and negatively correlated with the mean height of the vegetation cover and the temporal variability of indices of patchiness (Fig 3A). The second axis was positively correlated with both global variance and local covariance. The plots from the exclosure (S0) and the intensive grazing (S4) were clearly discriminated by the first axis. The characteristics of the vegetation under moderate grazing (S2) were intermediate between both. In particular, the plots from this grazing regime were dispersed along the second axis. The important dispersion of these plots in the factorial plan indicated that they differed in their global variance and local covariance, suggesting the existence of fine-grained patchiness in only some of them. On the contrary, plots from the intensive grazing regime (S4) were tightly aggregated and thus proved similar this respect.

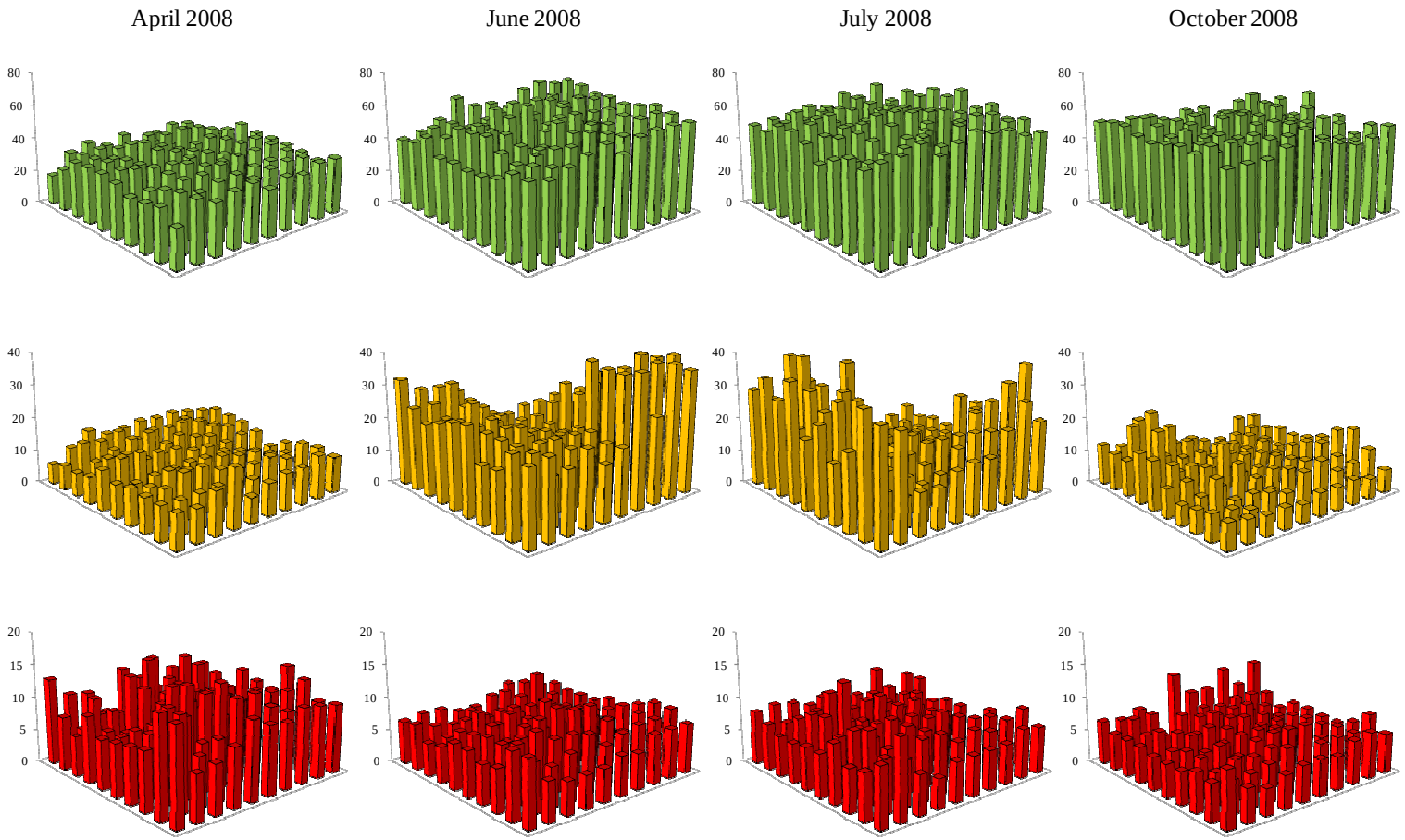

Fig. 2–Three dimensional representation of the vegetation height according to the grazing regime and the date. One representative grid per grazing regime is shown. Green: no grazing (S0), yellow: intermediate grazing (S2), red: heavy grazing (S4). The scale of the y-axis changes according to the grazing regime.

Moreover, their position along the second axis suggested low variance between cells of these plots, *i.e.* homogeneity rather than patchiness (Fig 3B).

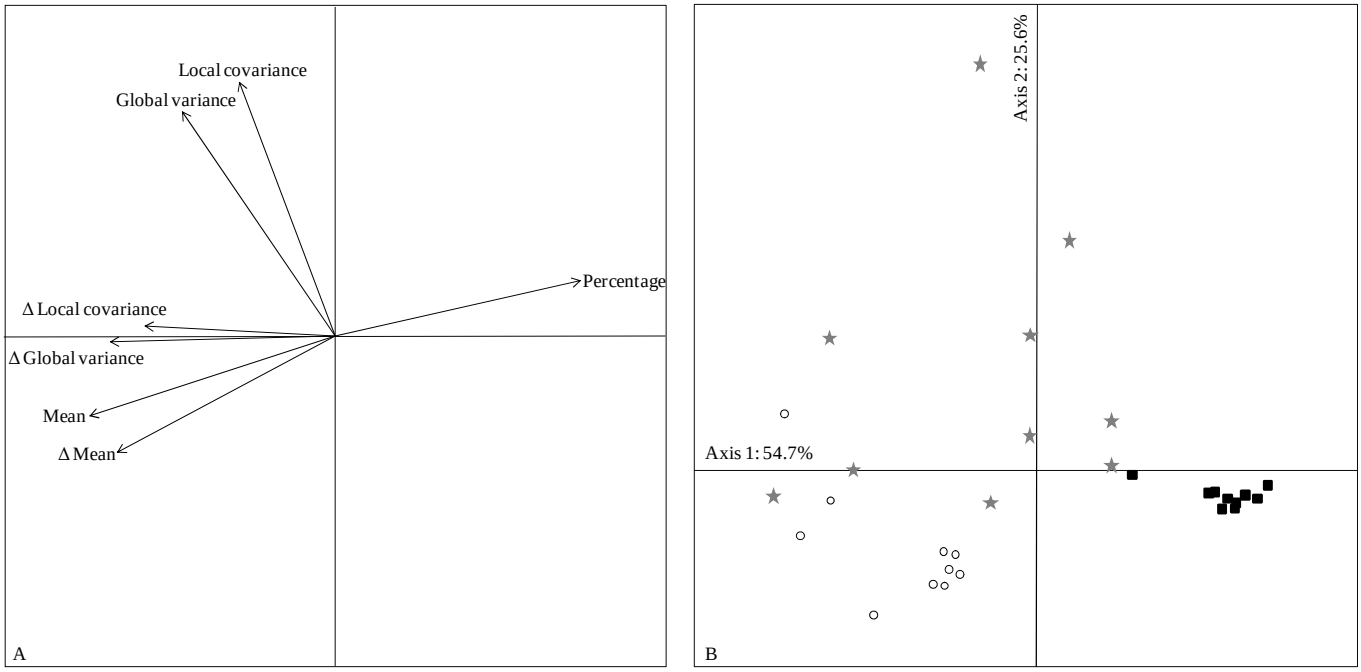

Fig. 3–Principal Component Analysis (PCA) between plots and spatio-temporal indices of the vegetation cover. (A) Factorial plan F1–F2 of indices. (B) Factorial plan F1–F2 of plots; open circles: no grazing (S0), grey stars: intermediate grazing (S2), black squares: heavy grazing (S4).

Relation between spatio-temporal indices and clonal traits

The co-inertia analysis showed 52 % of inertia shared by both matrices, indicating a strong relation between the characteristics of the vegetation height and clonal traits. This relation was significant (Monte-Carlo permutation test, $P < 0.001$).

A great majority of the inertia was explained by the first axis (95.5 %), which was positively correlated with the percentage of defoliated cells and negatively correlated with the mean height of the vegetation and the three indices of temporal variability (Fig 4A). The first axis was positively correlated with a moderate rate of clonal multiplication (CMR2-10), perennial ramets (ShLS2) and combinations of clonal traits and clonal growth organs characteristic of (i) non-clonal annual plants (Integ0, CMR0, Spr0 and CGO0), (ii) '*above-ground splitters*': big above-ground bud bank (BBAB) and small below-ground bud bank (BBBS), short-lived connections (Integ1) and stolons (CGO1), plantlets or plant fragments (CGO4-5), and (iii) tussock-forming clonal plants: short distance clonal spreading (Spr1) and short epigeogenous rhizomes (CGO9; Fig. 4B).

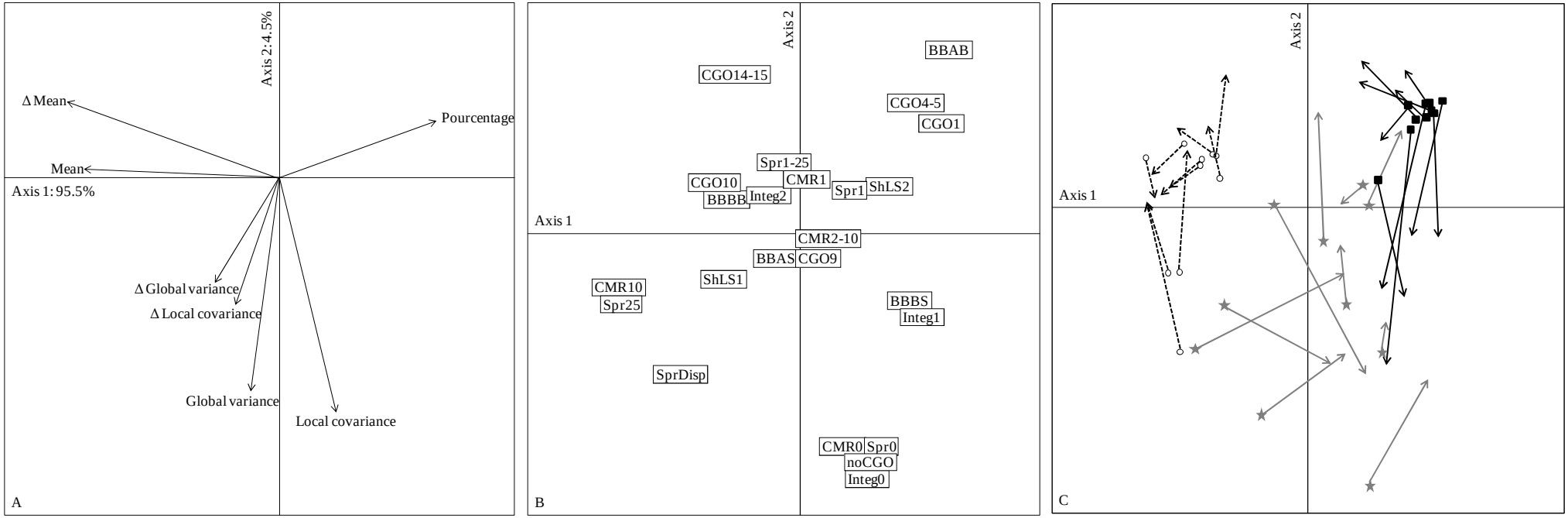

Fig 4–Co-inertia analysis between the matrices plots × spatio-temporal indices of the vegetation cover and plots × traits. (A) Factorial plan of the indices. (B) Factorial plan of the clonal traits. (C) Factorial plan of the plots. Plots are represented by arrows; arrow bases: according to the indices, arrow points: according to clonal traits.

By contrast, this axis was negatively correlated with a set of traits characterizing '*bellow-ground integrators*': small above-ground (BBAS) and big below-ground bud banks (BBBB), long-lived connections (Integ2), long hypogeogenous rhizomes (CGO10) and clonal growth organs originating from roots (CGO14-15), and with annual ramets (ShLS1), high rate of clonal multiplication (CMR10), high distance and free clonal spreading (Spr25 and SprDisp; Fig. 4B). The second axis of the co-inertia analysis explained only 4.5 % of the total inertia. It was negatively correlated with global variance and local covariance of the vegetation height (Fig 4A) and positively correlated with low clonal multiplication rate (CMR1) and moderate distance of lateral spread (Spr1-25; Fig. 4B). The first axis separated plots according to the grazing regime, while the plots from the moderate grazing regime were the most dispersed along the second axis (Fig. 4C).

Discussion

Impact of grazing on the spatio-temporal patterns of the vegetation height

As already shown on rabbit-grazed vegetation (Olofsson *et al.* 2008), vegetation height is a good estimator of cattle impact on vegetation: the more intensive the grazing regime, the shorter the canopy height. However, grazing has repeatedly been shown to favor species with a low stature (Diaz *et al.* 2001, 2007), as this trait provides plants with the ability to avoid defoliation (Briske 1996). Consequently, whether vegetation height translated grazing-induced defoliation or to long-term species responses to past grazing remains unclear and one can expect that it integrated both effects.

Our results provided evidence for a fine-grained spatial pattern of the vegetation height. Auto-correlograms indicated the existence of small-scale patches, from 0.2 m to 0.5 m-large according to the sampling date and the grazing regime, and randomness or homogeneity at higher scales. The existence of small-scale patches in the exclosure without grazing was the indication of a fine-grained patchiness due to intrinsic properties of the vegetation (plant species) or even to micro-environmental conditions (soil compaction, topography, water or nutrient availability; Rietkerk *et al.* 2000, Adler *et al.* 2001). In the exclosure, the size of patches increased from April to June and then decreased until October. Despite a similar trend under intensive grazing, smaller patches and non-significant Moran's I from 0.2 – 0.3 cm onwards, during the first months of the grazing season (April to June), suggested that intensive grazing tended to randomness or homogeneity of the vegetation

height. When the stocking rate increases, forage availability per herbivore decreases. Consequently, the vegetation is most completely exploited and the proportion of ungrazed patches decreases, notably because herbivores are compelled to feed on little palatable vegetation, which they would have otherwise avoided (Weber *et al.* 1998). At the end of the grazing season, patches in the exclosure and under intensive grazing were of similar size (0.2 m-large), suggesting that this fine-grained patchiness is not caused by grazing. By contrast, patches under moderate grazing were twice larger (0.4 m-large). Thus, moderate grazing appeared to enhance fine-grained patchiness.

In accordance with the results of auto-correlograms, both global variance and local covariance of vegetation height were rather weak estimators of the grazing regime (Fig. 3). In particular, they proved similar between plot of the exclosure without grazing (S0) and the intensive grazing (S4). However, plots from intensive grazing resembled each other, whereas a greater dissimilarity was recorded between plots from the exclosure. Consequently, intensive grazing tended to the homogenization of the vegetation cover, not only at a fine but especially at a large scale. By contrast, the great variation of spatio-temporal indices between plots from moderate grazing (Fig. 3) demonstrated that this grazing regime enhanced coarse-grained patchiness. Previous studies carried out in the same meadows have shown the existence of grazing-generated patch mosaics of vegetation structure and composition from 10 to several 100 m² (Loucougaray *et al.* 2004, Rossignol *et al.* 2006). Hierarchical, nested patterns have already been observed in soil properties of grazed meadows (Augustine & Frank 2001) and it is tempting to conclude that moderate grazing generated similar patterns of defoliation. However, while results of the auto-correlograms suggested an increase in fine-grained patchiness under moderate grazing, this phenomenon did not occur in all plots. On the contrary, global variance and local covariance of vegetation height were high in only a few plots from moderate grazing (Fig. 3). Indeed, grazing-induced defoliation primarily consists of discrete events at a bite-scale (Schwinning & Parsons 1999, WallisDeVries *et al.* 1999), *i.e.* ten centimeters or so, and could thus be expected to generate fine-grained patchiness. However, large mammalian herbivores such as cows can graze larger patches by simple head movements or a few steps (WallisDeVries *et al.* 1999). Our results provide evidence that, although cattle grazing could induce fine-grained patchiness, this phenomenon is rather rare. Given the size and selectivity of study herbivores, coarse-grained patchiness is more probable. Such patch grazing is expected to involve recurrent grazing of the same areas (Adler *et al.* 2001, Moussie *et al.* 2008), suggesting that large patches of vegetation are stable in time.

Unfortunately, our experimental design did not enable to estimate the inter-annual dynamics of finer-scale patterns of the vegetation cover.

The indices of temporal variation from April to October were negatively related to the grazing regime, which means that intensive grazing buffered intra-annual variation of the vegetation structure. In the exclosure, vegetation height was mainly governed by plant growth: the canopy was thus expected to be higher at the end of the grazing season, which corresponded to the end of the growing season. On the contrary, in grazed paddocks, vegetation growth, in particular of above-ground plant parts, might have been limited by defoliation (Ferraro & Oesterheld 2002). In this vegetation, dominated by grasses and graminoids, defoliation after culm elongation, which occurred mainly during the peak of vegetation (from May to July), might have prevented internode elongation and thus limited regrowth (Gold & Caldwell 1989). Such phenomenon likely occurred in our study site, where the vegetation was dominated by grasses and graminoids. Moreover, as mentioned above, this observation can also be explained by the small height of the vegetation under intensive grazing, the absolute variation of which may have been limited, compared to the vegetation of the exclosure.

Relation between spatio-temporal indices and clonal traits

Our results suggested that grazing-induced defoliation was unlikely to be perceived as patchy at the scale of the clonal fragment (*i.e.* between ten centimeters and one meter). As expected, neither spatial expansion nor extensive physiological integration, which we assumed to occur in fine-grained patchiness, were found in grazed areas. However, and contrary to our expectations, the observed coarse-grained patchiness did not clearly favored bud banks protected from defoliation, high clonal multiplication rates nor specialized storage organs. Indeed, grazing tended to promote above-ground splitters, tussock growth forms and even non-clonal annuals, while below-ground integrators dominated the ungrazed vegetation.

Clonal traits favored by grazing

First of all, grazing and particularly moderate grazing proved to favor non-clonal annual species. Such species, which rely only on sexual reproduction, have regularly been shown to take advantage of disturbances, particularly grazing (Lavorel *et al.* 1997, Diaz *et al.* 2007). A short phenological cycle would represent a mechanism to avoid grazing, in particular by spending grazing season as resistant forms such as seeds (Briske 1996). Moreover, by opening

gaps in the canopy, grazing herbivores could facilitate seed germination and efficient seedling establishment, which would have otherwise been prevented by competition for light due to canopy closure (Lavorel *et al.* 1997). Moreover, grazing could enhance the propagation of sexually reproducing species through endozoochory and epizoochory (Moussie *et al* 2005, Couvreur *et al.* 2008). Intermediate grazing regime seemed to be the modality where the abundance of annuals was the highest. Coarse-grained patchiness expressed at this grazing regime could be more beneficial to annuals. Plant individuals situated in less grazed patches were more likely to produce seeds that could germinate in gaps opened by defoliation. By contrast, homogeneous grazing could be more detrimental to sexual reproduction as it might regularly damage flowers and seeds (Fahrig *et al.* 1994).

In addition to the presence of non-clonal annuals, grazed vegetation was characterized by singular combinations of clonal traits. Whatever the grazing regime, short-lived connections and low distance lateral spreading were characteristic of grazed vegetation. These observations were confirmed by the dominance of above-ground clonal growth organs (stolons, plant fragments and plantlets) and short epigeogenous rhizomes in grazed paddocks. Neither extensive clonal integration among ramets, nor long distance lateral spreading was favored by grazing. Indeed, these traits would fail to enable the clonal fragment to avoid defoliation by escaping unfavorable, frequently grazed sites, as the size of these latter ones likely exceeded the size of the clonal fragment. Moreover the production and maintenance of long and physiologically functional connections are costly (van Groenendeal *et al.* 1996). In previous studies, mowing has been shown to limit rhizome increment and to disfavor lateral spread (Sammul *et al.* 2004, Gross *et al.* 2007). Similarly a little investment in the production of connections would expectedly be advantageous under grazed conditions where tissue losses and compensatory growth following defoliation may divert an important part of the resources.

We also expected storage functions to be involved in grazing tolerance as stored resources may enable the clonal fragment to buffer damages and to resume growth after defoliation (Iwasa & Kubo 1997). At first glance this property did not seem selected for in grazed areas, as none CGO assumed to be involved in resource storage (*e.g.* rhizomes, tubers; Suzuki & Stuefer 1999) were characteristic of the grazed vegetation. Similarly, the life span of the connections was rather short, limiting their ability to store and share resources. However, although expected to be mainly involved in spatial expansion (Dong & de Kroon 1994), stolons may have the potential to store resources. The connection to a fragment of stolon internode has been demonstrated to enhance the survival of young ramets separated

from the older ramets in *Potentilla anserina* (Stuefer & Huber 1999). Moreover, shoot bases have the ability to store resources (Cheplick & Chui 2001). Long lived ramets occurring in grazed paddocks may thus enable resource storage. These resources could be all the more efficiently remobilized as they are situated close to the damaged tissues.

Clonal traits in ungrazed conditions

The vegetation of the exclosure was characterized by below-ground integrators producing mainly long and long-lived below-ground connections but annual ramets. This observation should be considered with caution as it was partly driven by the dominance of one species, *Elytrigia repens*, while other species are present in lower abundances.

Long-distance clonal spreading has already been shown as a characteristic of abandoned grasslands (Tamm *et al.* 2002). This ability has been related to competitive ability (Grime 1977), as it would enable a clonal fragment to colonize space, thus preventing other species to establish. Long-lived below-ground organs are particularly efficient in resource storage (Dong & de Kroon 1994, Suzuki & Stuefer 1999). As suggested by the high rate of clonal multiplication observed in the vegetation of the exclosure, this property might enable the establishment of ramets despite competition, by supporting young ramets through resource retranslocation. These assumptions are in accordance with Goldberg and Landa's suggestion that early and fast growth from the emergence would enable efficient competitive responses (Goldberg & Landa 1991). By contrast dense canopy closure is likely to prevent seeds, plant fragments or stolon-borne ramets to efficiently establish.

Conlcusion

Our study provides evidence that grazing-induced defoliation was too coarse-grained to be perceived heterogeneously at the scale of the clonal fragment. Consequently, grazing did not promote clonal propertied associated with efficient responses to environmental patchiness. Grazing favored combinations of clonal traits enabling plants to cope with defoliation, mainly by minimizing the costs of clonal growth. By contrast, the absence of grazing favored clonal traits enhancing competitive ability. However, this study relies on potential traits that were documented in CLO-PLA3 database (Klimešová & Klimeš 2008). The measurement of clonal traits *in situ*, or in response to experimental defoliation could allow to evaluate the relevance of clonal properties highlighted by this study. The description of clonal growth forms in vegetation submitted to smaller herbivores, such as invertebrates, could go further previous

experimental studies (*e.g.* Stuefer *et al.* 2004, Gómez & Stuefer 2006, Gómez *et al.* 2007, 2008) and improve our knowledge on the involvement of clonal properties in resistance to multi-scale herbivory.

Literature cited

Adler PB, Raff DA & Laurenroth WK (2001) The effect of grazing on the spatial heterogeneity of vegetation. *Oecologia*, 128: 465-479.

Anderson TM, McNaughton SJ & Ritchie ME (2004) Scale-dependent relationships between the spatial distribution of a limiting resource and plant species diversity in an African grassland ecosystem. *Oecologia*, 139: 277-287.

Augustine DJ (2003) Spatial heterogeneity in the herbaceous layer of a semi-arid savanna ecosystem. *Plant Ecology*, 167: 319-332.

Augustine DJ & Frank DA (2001) Effects of migratory grazers on spatial heterogeneity of soil nitrogen properties in a grassland ecosystem. *Ecology*, 82: 3149-3162.

Bakker JP, de Leeuw J & van Wieren E (1983) Micro-patterns in grassland vegetation created and sustained by sheep-grazing. *Vegetatio*, 55: 153-161.

Bakker C, Blair JM & Knapp AK (2003) Does resource availability, resource heterogeneity or species turnover mediate changes in plant species richness in grazed grasslands? *Oecologia*, 137, 385-391.

Bell AD & Tomlinson PB (1980) Adaptive architecture in rhizomatous plants. *Botanical Journal of the Linnean Society*, 80: 125-160.

Bell TL & Ojeda F (1999) Underground starch storage in *Erica* species of the Cape Floristic Region – differences between seeders and resprouters. *New Phytologist*, 144: 143-152.

Bellingham PJ & Sparrow AD (2000) Resprouting as a life history strategy in woody plant communities. *Oikos*, 89: 409-416.

Briske DD (1996) Strategies of plant survival in grazed systems: a functional interpretation. In: Hodgson J & Illius AW (eds) *The Ecology and management of grazing systems*. CAB International, Wallingford, pp 33-67.

Cain ML (1994) Consequences of foraging in clonal plant species. *Ecology*, 75: 933-944.

Cheplick GP & Chui T (2001) Effect of competitive stress on vegetative growth, storage, and regrowth after defoliation in *Phleum pratense*. *Oikos*, 95: 291-299.

Chessel D, Dufour AB & Thioulouse J (2004) The ade4 package – I – One-table methods. *R News*, 4: 5-10.

Cliff AD & Ord JK (1973) *Spatial autocorrelation.* Pion, London.

Cliff AD & Ord JK (1981) *Spatial processes: models and applications.* Pion, London.

Collins SL & Smith MD (2006) Scale-dependent interaction of fire and grazing on community heterogeneity in tallgrass prairie. *Ecology*, 87: 2058-2067.

Couvreur M, Verheyen K, Vellend M, Lamoot I, Cosyns E, Hoffmann M & Hermy M (2008) Epizoochory by large herbivores: merging data with models. *Basic and Applied Ecology*, 9: 204-212.

D'Hertefeld T & Falkengren-Grerup U (2002) Extensive physiological integration in *Carex arenaria* and *Carex disticha* in relation to potassium and water availability. *New Phytologist*, 156: 469-477.

D'Hertefeldt T & Jónsdóttir IS (1999) Extensive physiological integration in intact clonal systems of *Carex arenaria*. *Journal of Ecology*, 87: 258-264.

de Bello F, Leps J & Sebastià MT (2005) Predictive value of plant traits to grazing along a climatic gradient in the Mediterranean. *Journal of Applied Ecology*, 42: 824-833.

de Kroon H & Schieving F (1990) Resource partitioning in relation to clonal growth strategy. In: van Groenendael J & de Kroon H (eds) *Clonal growth in plants: regulation and function*. SPB Academic Publishing, The Hague, pp 113-130.

Diaz S, Lavorel S, McIntyre S, Falczuk V, Casanoves F, Michunas DG, Skarpe C, Rusch G, Sternberg M, Noy-Meir I, Landsberg J, Zhang W, Clark H & Campbell BD (2007) Plant trait responses to grazing – a global synthesis. *Global Change Biology*, 13: 313-341.

Diaz S, Noy-Meir I & Cabido M (2001) Can grazing response of herbaceous plants be predicted from simple vegetative traits? *Journal of Applied Ecology*, 38: 497-508.

Dong M & de Kroon H (1994) Plasticity in morphology and biomass allocation in *Cynodon dactylon*, a grass species forming stolons and rhizomes. *Oikos*, 70: 99-106.

Dong M & Pierdominici MG (1995) Morphology and growth of stolons and rhizomes in three clonal grasses, as affected by different light supply. *Vegetatio*, 116: 25-32.

Fahrig L, Coffin DP, Lauenroth WK & Shugart HH (1994) The advantage of long-distance spreading in highly disturbed habitats. *Evolutionary Ecology*, 8: 172-187.

Ferraro DO & Oesterheld M (2002) Effect of defoliation on grass growth. A quantitative review. *Oikos*, 98: 125-133.

Fischer M & van Kleunen M (2002) On the evolution of clonal plant life histories. *Evolutionary Ecology*, 15: 565-582.

Gold WG & Caldwell MM (1989) The effects of the spatial pattern of defoliation on regrowth of a tussock grass. I. Growth responses. *Oecologia*, 80: 289-296.

Goldberg DE & Landa K (1991) Competitive effect and response: hierarchies and correlated traits in the early stages of competition. *Journal of Ecology*, 79: 1013-1030.

Gómez S & Stuefer JF (2006) Members only: induced systemic resistance to herbivory in a clonal plant network. *Oecologia*, 147: 461-468.

Gómez S, Latzel V, Verhulst YM & Stuefer JF (2007) Costs and benefits of induced resistance in a clonal plant network. *Oecologia*, 153: 921-930.

Gómez S, Onoda Y, Ossipov V & Stuefer JF (2008) Systemic induced resistance: a risk-spreading strategy in clonal plant networks? *New Phytologist*, 179: 1142-1153.

Grime JP (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist*, 111: 1169-1194.

Gross N, Suding K & Lavorel S (2007) Leaf dry matter content and lateral spread predict response to land use change for six subalpine grassland species. *Journal of Vegetation Science*, 18: 289-300.

Hartnett DC (1989) Density- and growth stage-dependent responses to defoliation in two rhizomatous grasses. *Oecologia*, 80: 414-420.

Herms DA & Mattson WJ (1992) The dilemma of plants: to grow or defend. *Quarterly Review of Biology*, 67: 283–335.

Huntly N (1991) Herbivores and the dynamic of communities and ecosystems. *Annual Review of Ecology and Systematics*, 22: 477-503.

Hutchings MJ (1999) Clonal plants as cooperative systems: benefits in heterogeneous environments. *Plant Species Biology*, 14: 1-10.

Hutchings MJ & Bradbury IK (1986) Ecological perspectives on clonal perennial herbs. *BioScience*, 36: 178-182.

Hutchings MJ & Mogie M (1990) The spatial structure of clonal plants: control and consequences. In: van Groenendael J & de Kroon H (eds) *Clonal growth in plants: regulation and function*. SPB Academic Publishing, The Hague, pp 57-78.

Iwasa Y & Kubo T (1997) Optimal size of storage for recovery after unpredictable disturbances. *Evolutionary Ecology*, 11: 41-65.

Jónsdóttir IS & Callaghan TV (1989) Localized defoliation stress and the movement of ^{14}C-photoassimilates between tillers of *Carex bigelowii*. Oikos, 54: 211-219.

Jónsdóttir IS & Watson MA (1997) Extensive physiological integration: an adaptive trait in resource-poor environments? In: de Kroon H & van Groenendael J (eds) *The ecology and evolution of clonal plants*. Backhuys Publishers, Leiden, pp 109-136.

Jónsdóttir IS, Augner M, Fagerström T, Persson H & Stenström (2000) Genet age in marginal populations of two clonal *Carex* species in the Siberian Arctic. *Ecography*, 23: 402-412.

Kelly CK (1995) Thoughts on clonal integration: facing the evolutionary context. *Evolutionary Ecology*, 9: 575-585.

Klimeš L, Klimešovà J, Hendricks R & van Groenendael J (1997) Clonal plant architecture: a comparative analysis of form and function. In: de Kroon H & van Groenendael J (eds) *The ecology and evolution of clonal plants*. Backhuys Publishers, Leiden, pp 1-29.

Klimešovà J & de Bello F (2009) CLO-PLA: the database of clonal and bud bank traits of Central European flora. *Journal of Vegetation Science*, 20: 511-516.

Klimešová J & Klimeš L (2003) Resprouting of herbs in disturbed habitats: is it adequately described by Bellingham – Sparrow's model? *Oikos*, 103: 225-229.

Klimešovà J & Klimeš L (2007) Bud banks and their role in vegetative regeneration – A literature review and proposal for simple classification assessement. *Perspectives in Plant Ecology, Evolution and Systematics*, 8: 115-129.

Klimešovà J & Klimeš L (2008) Clonal growth diversity and bud banks of plants in the Czech flora: an evaluation using the CLO-PLA3 database. *Preslia*, 80: 255-275.

Kun A & Oborny B (2003) Survival and competition of clonal plant populations in spatially and temporally heterogeneous habitats. *Community Ecology*, 4: 1-20.

Lattanzi FA, Schnyder H & Thornton B (2004) Defoliation effects on carbon and nitrogen substrate import and tissue-bound efflux in leaf growth zones of grasses. *Plant, Cell and Environment*, 27: 347-356.

Lavorel S, McIntyre S, Landsberg J & Forbes TDA (1997) Plant functional classifications: from general groups to specific groups based on response to disturbance. *Trends in Ecology and Evolution*, 12: 474-478.

Loucougaray G, Bonis A & Bouzillé JB (2004) Effects of grazing by horses and/or cattle on the diversity of coastal grasslands in western France. *Biological Conservation*, 116: 59-71.

Lovett-Doust L (1981) Population dynamics and local specialization in a clonal perennial (*Ranunculus repens*). I. The dynamics of ramets in contrasting habitats. *Journal of Ecology*, 69:743-755.

Macek P, Lepš J (2003) The effect of environmental heterogeneity on clonal behaviour of *Prunella vulgaris* L. *Plant Ecology*, 168: 31-43.

McLellan AJ, Prati D, Kaltz O & Schmid B (1997) Structure and analysis of phenotypic and genetic variation in clonal plants. In: de Kroon H & van Groenendael J (eds) *The ecology and evolution of clonal plants*. Backhuys Publishers, Leiden, pp 185-210.

Ménard C, Ducan P, Fleurance G, George JY & Lila M (2002) Comparative foraging and nutrition in horses and cattle in Europe wetlands. *Journal of Applied Ecology*, 38: 120-133.

Moussie AM, Vos P, Verhagen HMC & Bakker JP (2005) Endozoochory by free-ranging, large herbivores: ecological correlates and perspectives for restoration. *Basic and Applied Ecology*, 6: 547-558.

Moussie AM, Apol MEF, Heil GW & van Diggelen R (2008) Creation and preservation of vegetation patterns by grazing. *Ecological Modelling*, 218: 60-72.

Olofsson J, de Mazancourt C & Crawley MJ (2008) Spatial heterogeneity and plant species richness at different spatial scales under rabbit grazing. *Oecologia*, 156: 825-834.

Oom SP, Sibbald AM, Hester AJ, Miller DR & Legg CJ (2008) Impacts of sheep grazing a complex vegetation mosaic: relating behavior to vegetation change. *Agriculture, Ecosystems and Environment*, 124: 219-228.

Pakeman RJ (2004) Consistency of plant species and trait responses to grazing along a productivity gradient: a multi-site analysis. *Journal of Ecology*, 92: 893-905.

Pakeman RJ, Pywell RF & Wells TCE (2002) Species spread and persistence: implications for experimental design and habitat re-creation. *Applied Vegetation Science*, 5: 75-86.

Pitelka LF & Ashmun JW (1985) Physiology and integration of ramets in clonal plants. In: Jackson JBC, Buss LW & Cook RE (eds) *Population biology and evolution of clonal organisms*. Yale University Press, New Haven, pp 399-435.

Posse G, Anchorena J & Collantes MB (2000) Spatial micro-patterns in the steppe of Tierra del Fuego induced by sheep grazing. *Journal of Vegetation Science*, 11: 43-50.

Puijalon S, Bouma TJ, van Groenendael J & Bornette G (2008) Clonal plasticity of aquatic plant species submitted to mechanical stress: escape versus resistance strategy. *Annals of Botany*, 102: 989-996.

R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Richards JH (1993) Physiology of plants recovering from defoliation. In: Baker MJ (ed) *Proceedings of the XVII International Grassland Congress*. SIR Publishing, Wellington, pp 85-94.

Rietkerk M, Ketner P, Burger J, Hoorens B & Olff H (2000) Multiscale soil and vegetation patchiness along a gradient of herbivore impact in a semi-arid grazing system in West-Africa. *Plant Ecology*, 148: 207-224.

Rossignol N, Bonis A & Bouzillé JB (2006) Relationships between grazing pattern and vegetation structure on the spatial variations of net N mineralisation in a wet grassland. *Applied Soil Ecology*, 31: 62-72.

Sammul M, Kull K, Niitla T & Möls T (2004) A comparison of plant communities on the basis of their clonal growth patterns. *Evolutionary Ecology*, 18: 443-467.

Schwinning S & Parsons AJ (1999) The stability of grazing system revisited: spatial models and the role of heterogeneity. *Functional Ecology*, 13: 737-747.

Sokal RR & Oden NJ (1978) Spatial autocorrelation in biology 1. Methodology. *Biological Journal of the Linnean Society*, 10: 199-228.

Stuefer JF (1996) Potential and limitations of current concepts regarding the response of clonal plants to environmental heterogeneity. *Vegetatio*, 127: 55-70.

Stuefer JF & Huber H (1999) The role of stolon internodes for ramet survival after clone fragmentation in *Potentilla anserina*. *Ecology Letters*, 2: 135-139.

Stuefer JF, Gómez S & van Mölken T (2004) Clonal integration beyond resource sharing: implications for defence signalling and disease transmission in clonal plant networks. *Evolutionary Ecology*, 18: 647-667.

Suzuki JI & Stuefer J (1999) On the ecological and evolutionary significance of storage in clonal plants. *Plant Species Biology*, 14: 11-17.

Tamm A, Kull K & Sammul M (2002) Classifying clonal growth forms based on vegetative mobility and ramet longevity: a whole community analysis. *Evolutionary Ecology*, 15: 383-401.

van Groenendael JM, Klimeš L, Klimešovà J & Hendricks R (1996) Comparative ecology of clonal plants. *Philosophical Transactions of the Royal Society London*, 351: 1331-1339.

Veen GF, Blair JM, Smith MD & Collins SL (2008) Influence of grazing and fire frequency on small-scale plant community structure and resource variability in native tallgrass prairie. *Oikos*, 117: 859-866.

WallisDeVries MF, Laca EA & Demment MW (1999) The importance of scale of patchiness for selectivity in grazing herbivores. *Oecologia*, 121: 355-363.

Weber GE, Jeltsch F, van Rooyen N & Milton SJ (1998) Simulated long-term vegetation response to grazing heterogeneity in semi-arid rangelands. *Journal of Applied Ecology*, 35: 687-699.

Westoby M (1998) A leaf-height-seed (LHS) plant ecology strategy scheme. *Plant and Soil*, 199: 213-227.

Wijesinghe DK & Hutchings MJ (1997) The effects of spatial scale of environmental heterogeneity on the growth of a clonal plant: an experimental study with *Glechoma hederacea*. *Journal of Ecology*, 85: 17-28.

Wijesinghe DK & Hutchings MJ (1999) The effects of environmental heterogeneity on the performance of *Glechoma hederacea*: the interactions between patch contrast and patch scale. *Journal of Ecology*, 87: 860-872.

Wolfer SR, van Nes EH & Straile D (2006) Modelling the clonal growth of the rhizomatous macrophyte *Potamogeton perfoliatus*. *Ecological Modelling*, 192: 67-82.

Zhou Z, Sun OJ, Luo Z, Jin H, Chen Q & Han X (2008) Variation in small-scale spatial heterogeneity of soil properties and vegetation with different land use in semiarid grassland ecosystem. *Plant Soil*, 310: 103-112.

Article 3 – Are clonal traits and plastic responses to defoliation good indicators of grazing resistance? A test on eight clonal species.

Marie-Lise Benot[1], Cendrine Mony[1], Jan Lepš[2] & Anne Bonis[1]

[1]UMR CNRS 6553, ECOBIO, Université de Rennes 1, Campus de Beaulieu, 35042 Rennes Cedex, France

[2]Department of Botany, Faculty of Science, University of South Bohemia, Na Zlaté stoce 1, 370 05 Ceske Budejovice, Czech Republic

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Abstract

Grazing is expected to act as a filter on plant traits, promoting traits that enable the plant to develop and reproduce under the conditions it generates (response traits). Grazing resistance can be divided into strategies of avoidance and tolerance. Most studies dealing with functional responses to grazing concentrate on morphological and reproductive traits. Despite the great diversity of clonal growth forms, clonal traits are generally neglected. Our objective was to test the hypothesis that grazing resistance involves clonal traits, depending on both species-specific traits and trait responses to defoliation. In that purpose, we first estimated the level of grazing resistance in eight clonal species. Secondly, we analyzed the relationship between grazing resistance and (i) clonal traits measured in undisturbed conditions and (ii) their responses to experimental defoliation. Grazing resistance was negatively related to vegetative height and biomass of below-ground clonal organs, and positively related to the response of both traits to defoliation. These traits can be associated with a balance between avoidance and competitiveness. A short stature limits the amount of biomass removed by herbivores, while a high canopy promotes above-ground competition. A large investment in below-ground clonal organs, which generally serve as storage structures, may enhance competitive ability, notably by supporting vegetative multiplication despite canopy closure. We concluded that defoliation tolerance was not the dominant strategy of resistance in the study meadow. Moreover, clonal traits were poor indicators of grazing resistance.

Key words

Avoidance; competitiveness; graminoids; grasses; plastic responses to defoliation; redundancy analysis (RDA); tolerance.

Introduction

Herbivory often induces changes in the structure and the composition of plant meadow communities (McNaughton 1979, Belsky 1987, Milchunas *et al*. 1988, Huntly 1991, Bullock *et al*. 2001). Species have traditionally been classified on the basis of their level of resistance to herbivory. Indeed, resistant species, which become more abundant in response to grazing, are often referred to as increasers, whereas sensitive species, which become scarcer when grazed, correspond to decreasers (Dykserhuis 1949, Diaz *et al*. 2001, Vesk & Westoby 2001, del-Val & Crawley 2004, 2005). Grazing is expected to promote traits that enable plant survival, development and reproduction in the conditions it generates (*i.e.* response traits; Keddy 1992, Diaz *et al*. 1998, Lavorel & Garnier 2002). Several studies attempted to link grazing-induced changes in plant communities with plant functional traits, aiming to explain and predict these changes (de Bello *et al*. 2005, Diaz *et al*. 2007). Such studies consider the overall impacts of grazing on plants, including both direct (damages to plant tissues) and indirect effects (modifications of plant biotic and abiotic environment) (Harper 1977, Diaz *et al*. 2001).

As proposed by Bullock *et al*. (2001), the study of species responses to single grazing components (*e.g.* tissue removal, trampling, urine and feces deposition) would likely clarify the understanding of grazing-induced changes in plant communities. In cattle-grazed meadows, the removal of above-ground tissues (here onwards referred to as *defoliation*) has been suggested as the most important grazing process influencing plant community composition (Kohler *et al*. 2004). In particular, grazing resistance can be associated with either defoliation avoidance (*i.e.* mechanisms that decrease the probability of being defoliated) or tolerance (*i.e.* mechanisms that enhance growth and reproduction after defoliation; Briske 1996). In almost all cases, plant growth form and plant height have been highlighted as the best predictors of plant response to grazing (Noy-Meir *et al*. 1989, Briske & Silvertown 1993, Diaz *et al*. 2001, 2007), as they govern the balance between competition and avoidance strategies. However, in grazed systems, particularly those under intensive management, the odds escaping defoliation may be low, and avoidance mechanisms are likely to be overcome (Richards 1993). In such situations, plant species that are able to tolerate for tissue losses may be of great advantage (Stowe *et al*. 2000). A handful of studies investigated the relation between defoliation and grazing responses. They confirmed that grazing resistance in the field was associated with tolerance to defoliation (del-Val & Crawley 2004, 2005): while decreasers hardly regrew after defoliation, increasers often proved able to compensate for tissue losses even after intense and frequent clipping.

Functional responses to grazing have generally been described on the basis of life history, plant growth form and rough morphological traits, principally because they are easy to measure (Diaz *et al* 2001, Weiher *et al*. 1999). Although disturbance has been recognized to favor annual species (Grime 1977, Lavorel *et al*. 1997, Diaz *et al*. 2007), vegetative multiplication (*i.e.* clonal growth) constitutes the main form of reproduction and population persistence in many grasslands (Briske & Silvertown 1993). Clonal growth forms are diverse and the expression of clonal traits varies according to environmental conditions (Klimeš *et al*. 1997, Tamm *et al*. 2002, Sammul *et al*. 2004, Halassy *et al*. 2005). Yet, clonal growth remains poorly considered in studies dealing with plant functional traits and responses to defoliation, and it is often reduced to the clonal growth form (*e.g.* stoloniferous *vs.* tussock forming, Diaz *et al*. 2007).

Focusing on morphological, notably clonal traits (*i.e.* traits related to clonal structures and clonal multiplication), we aimed to determine the relative involvement of (i) species-specific traits and (ii) trait responses to defoliation, in the level of grazing resistance in eight clonal species. We particularly tested the hypothesis that grazing resistance would depend on both:

(i) Species-specific values of traits. We expected an increase in grazing resistance to be associated with a shift from traits conferring a high competitive ability to traits related to grazing resistance (avoidance or tolerance).

(ii) Trait response to defoliation. We expected an increase in grazing resistance to be associated with plastic responses of clonal traits that enhance tolerance to defoliation.

To test these hypotheses, we proceeded into two steps. First, we determined the level of grazing resistance of eight clonal species, on the basis of their shifts of abundance under contrasted grazing regimes. Secondly, we experimentally assessed clonal trait values in undisturbed conditions and clonal trait responses to defoliation in these eight species.

Material and methods

Study site and vegetation sampling

The study site is located in the Marais Poitevin, which is the second most important wetland of France (120,000 ha). It is located on French Atlantic coast (46° 28'N; 1° 13'W) and composed of wet meadows, which were reclaimed on the sea between the 10th and the 12th centuries.

The study was carried out on the grassland of Magnils-Reigniers (250 ha) where an experimental design was set up in 1995 to investigate the consequences of grazing scenarios on plant communities patterns (Rossignol *et al*. 2006). Field observations were conducted on mesophilous vegetation in three cattle-grazed paddocks differing in the grazing regime applied since 1995. The exclosure (S0) was a 4 ha paddock, from which grazing has been excluded since 1995. The two grazed paddocks were 1 ha-large. The intermediate (S2) and the highest (S4) stocking rates corresponded respectively to 2 cattle.ha^{-1} and 4 cattle.ha^{-1} (*i.e.* about 685 kg.ha^{-1} and 1370 kg.ha^{-1}, Ménard *et al*. 2002).

We sampled vegetation in June 2008, *i.e.* during the peak of vegetation, when most of the plant species were expected to be represented in the community. For each stocking rate (S0, S2 and S4), we recorded the relative percentage cover of each vascular plant species and of bare ground in ten 0.5 m × 0.5 m plots. All of the ten plots were randomly positioned in the paddock. Although this device can be regarded as pseudo-replication, we aimed to evaluate long-term effect of grazing on vegetation. Given the relatively ancient installation of the experimental design, we expected differences between paddocks to be driven by the grazing regime rather than other environmental factors.

Garden experiment

We established an experiment on eight clonal perennial Monocotyledons, which dominated the vegetation cover whatever the stocking rate (from 72 % for the intermediate stocking rate S2, to 98 % for the exclosure S0). We aimed to record their morphological and clonal traits both in undisturbed conditions and in response to defoliation. These species presented diverse clonal growth forms: clonal growth could be achieved either through the production of plagiotropic above-ground or below-ground stem-derived connections (*stoloniferous* and *rhizomatous* growth forms, respectively), or through the production of very short, rather inexistent connections (*tussock* growth from). A single species could combine several clonal growth forms (Table 1). In the present experiment, a clonal fragment consisted of the set of clonally-produced propagules (*tillers*) and *connections*.

Clonal fragments were collected in November 2006. As we aimed to measure the range of species-specific clonal trait values and responses to defoliation, we wanted to buffer phenotypic and/or genotypic variations potentially induced by carry-over effects (*i.e.* transmission of environmentally induced phenotypic changes to future generation, Schwaegerle *et al*. 2000) or local adaptations to grazing (*i.e.* selection of traits adapted to local conditions leading to genetically differentiated populations, Sultan & Spencer 2002).

Consequently, we randomly picked up about 20 fragments of each species in the 250 ha-large grassland of the Magnils-Reigner, where grazing regime is spatially variable. We paid attention to sample these fragments at a minimal distance of 5 m from each other. Collected plants were then grown for 5 months in rich garden soil in the experimental garden of University of Rennes (France).

Table 1 – List of studied species, their family and main and secondary clonal growth form (adapted from field and experimental observation, and CLOPLA-3 database, Klimešovà & Klimeš 2008, Klimešovà & de Bello 2009).

		Clonal growth form	
Species	Family	Main	Secondary
Agrostis stolonifera L.	Poaceae	Stoloniferous	Tussock
Cynosurus cristatus L.	Poaceae	Tussock	/
Carex divisa Huds.	Cyperaceae	Rhizomatous	/
Elytrigia repens L.	Poaceae	Rhizomatous	Tussock
Hordeum secalinum Schreb.	Poaceae	Tussock	/
Juncus gerardii Lois.	Juncaceae	Rhizomatous	/
Lolium perenne L.	Poaceae	Tussock	Stoloniferous
Poa trivialis L.	Poaceae	Tussock	Stoloniferous

We set up the experiment during a five-day period from 29 April to 3 May 2007. We selected 14 fragments of each species, from which we isolated three tillers. Each ramet was planted in the centre of a pot (0.2 cm in diameter, 16 cm in height) containing rich garden soil. We tested three defoliation treatments: no defoliation (control treatment), 12 cm-high defoliation (moderate treatment) and 6 cm-high defoliation (severe treatment). We randomly assigned a modality of the defoliation treatment to each of the three tillers belonging to a same fragment. Defoliation consisted of cutting above-ground shoots of tillers and non-rooted stolons above the cutting height. Clipped tissues were dried at 60°C to constant mass and weighed. We applied the first defoliation after a 21-day acclimation period during which tillers were allowed to root and grow. Defoliation events then occurred every 21 days, from 28 May to 30 July 2007. Defoliated plants were thus cut four times. The recovery period after the last defoliation event lasted for 21 days, and plants were harvested on 20 August 2007.

At the end of the experiment, we monitored morphological and clonal traits (Table 2). At harvest, we measured the vegetative height of plants. We then cleaned the individual plants and separated them into tiller shoots, stem bases (*i.e.* the below-ground part of tiller shoots), connections (stolons or rhizomes), roots and flowers. These organs were dried at 60°C to constant mass and weighed.

Table 2 – Morphological and clonal traits monitored at the end of the experiment

Trait	Measurement
Total biomass	Sum of the biomasses of all organs, including clipped tissues
Above-ground biomass	Biomass of tiller shoots, stolons, and clipped tissues
Below-ground biomass	Biomass of clonal organs situated below the ground surface (rhizomes and shoot bases)
Root biomass	Biomass of roots
Flower biomass	Biomass of flowers produced during the experiment
Number of tillers	Final number of tillers
Number of flowers	Number of flowers produced during the experiment
Number of connections	Final number of rhizomes or stolons
Distance of clonal spread	Distance from the parent ramet to the last node of the longest connection, or radius of the tussock
Vegetative height	Height of the highest vegetative tiller

Data analyses

In order to focus on the eight species studied in the experiment, we removed all other species from the abundance matrix species × plot (matrix A). We then recalculated the percentage cover of each species, so that the sum of abundances for each plot was 1 (matrix B, Pakeman 2004). We evaluated the level of grazing resistance for each species by applying redundancy analysis (RDA) on the resultant matrix B, with the stocking rate as the explanatory variable. The level of grazing resistance corresponded to the score of the species on the constrained axis of the RDA (de Bello *et al.* 2005).

For each species, we aimed to describe (i) the constitutive (*i.e.* species-specific) values of clonal traits, and (ii) the responses of clonal traits to defoliation. For that purpose, we used data extracted from the garden experiment. We considered the mean trait values in the control treatment (*i.e.* without defoliation) as the estimator of species-specific trait values. In order to assess trait response to defoliation, the defoliation treatment was expressed as an ordinal variable (0 = control, 1 = moderate and 2 = severe defoliation). For each species and each trait, we calculated the Spearman's rank correlation coefficient (ρ) between treatment and trait value. We thus obtained the score of each species in response to grazing (RDA score), and, for each trait, the species-specific mean trait value (i) and the species-specific response of the trait to defoliation (ii). We finally determined the importance of (i) traits and (ii) of their response to defoliation as predictors of species response to grazing. In this purpose, we tested for each trait, the Pearson's correlation coefficient (r) between the species RDA scores and (i)

the species-specific mean trait values, and (ii) the species-specific trait responses to defoliation.

RDA analysis was carried out using CANOCO (ter Braak & Šmilauer 1998). ANOVAs, post-hoc TukeyHSD tests, the calculation of correlation coefficient and associated tests were carried out with R software (R Development Core Team, 2007, http://www.R-project.org).

Results

Species status in the field: response to grazing

Species differed in their level of grazing resistance. *Elytrigia repens* was the only species with a negative score, indicated that its abundance was negatively related to the grazing regime. The scores of *Juncus gerardii*, *Carex divisa* and *Agrostis stolonifera* were comprised between 0 and 0.2. Their abundance was little affected by the grazing regime. *Poa trivialis*, *Hordeum secalinum*, *Cynosurus cristatus* and *Lolium perenne* were absent from the exclosure. Their abundances increased with the grazing regime as indicated by their scores higher than 0.5 (Fig. 1).

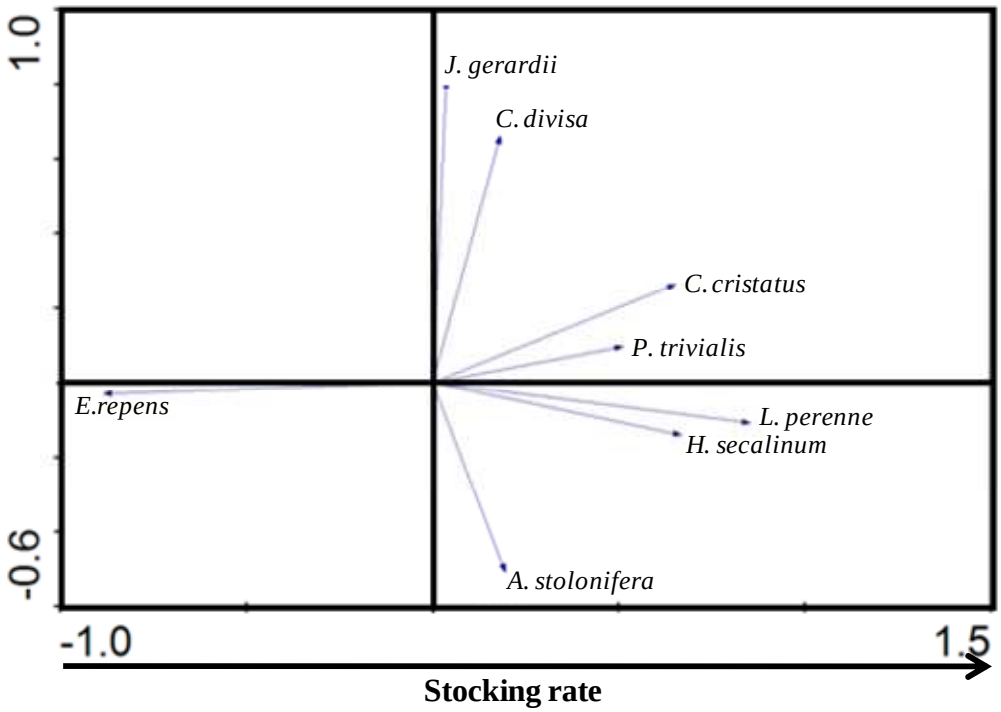

Fig. 1. Factorial plan F1 – F2 of the RDA on the species×plot matrix (matrix B), with the stocking rate as constraining factor.

Correlation between grazing resistance and species-specific traits

Pearson's correlation coefficient showed that only two traits were significantly related to grazing resistance. Grazing resistance was negatively correlated with both the mean biomass of below-ground organs and the mean vegetative height (Table 3).

Correlation between grazing resistance and trait response to defoliation

The grazing resistance was positively correlated to the response to defoliation only for vegetative height (Table 3). Grazing resistance tended to be positively correlated to the response of the biomass of below-ground organs (Table 3), although this correlation was only marginally significant ($P = 0.052$).

Table 3 – Values of Pearson's correlation coefficients between species scores in response to grazing and (i) species mean trait values, and (ii) species responses to defoliation for each trait, and related *P*-values. In bold: significant *P*-values.

Clonal trait	Species means		Species responses to defoliation	
	r	*P*	*r*	*P*
Total biomass	-0.15	0.732	0.38	0.359
Above-ground biomass	-0.05	0.906	0.27	0.515
Below-ground biomass	-0.91	**0.002**	0.70	**0.052**
Root biomass	-0.10	0.810	0.48	0.225
Flower biomass	0.53	0.172	-0.23	0.589
Number of tillers	0.08	0.855	-0.45	0.265
Number of flowers	0.56	0.150	-0.02	0.961
Number of connections	-0.18	0.675	0.18	0.664
Distance of clonal spread	-0.34	0.409	0.02	0.971
Vegetative height	-0.91	**0.002**	0.80	**0.017**

Discussion

Two traits (*i.e.* maximal vegetative height and biomass of below-ground organs) were related to species resistance to grazing. For both traits, the mean species-specific value, as well as the response to defoliation was related to species response to grazing. Our results suggest that, in the study plant community, grazing tended to favor strategies of defoliation avoidance rather than tolerance. Among clonal traits, only the biomass of below-ground organs was related to clonal growth. This indicates that, contrary to our expectations, clonal traits are poor indicators of species resistance to grazing.

Competition vs. avoidance, rather than tolerance

The ability to tolerate tissue losses is considered to be widespread among plants and notably, expected to be selected for in vegetation submitted to herbivores (Stowe *et al*. 2000). In a study on eight grassland species, del-Val & Crawley (2005) demonstrated that grazing resistance was significantly related to the ability of the species to compensate for losses of biomass caused by experimental defoliation. They concluded that tolerance to herbivory was an important factor influencing the structure of the vegetation in grazed environments. In the present study, the response of total plant biomass to defoliation (*i.e.* compensation) was not correlated to species level of grazing resistance. Moreover, for both the maximal vegetative height and the biomass of below-ground organs, response to defoliation depended on the initial value of the trait. The higher the initial value of the trait in undefoliated conditions, the more negatively it was impacted by defoliation. The ability of studied species to tolerate for defoliation thus proved weak and little involved in grazing resistance.

Our results proved that species the most resistant to grazing were shorter and allocated less biomass in below-ground organs than sensitive species. Plant growth form and height frequently emerged as the best predictors of species response to grazing: short statures and prostrate or rosette architectures proved to be characteristics of increasers in several systems (Noy-Meir *et al*. 1989, Diaz *et al*. 2001, 2007). By contrast, high canopies have been suggested as attributes of competitive plants (Grime 1977). In our study site, the exclosure without grazing is dominated by *E. repens*. The dense canopy generated by this species is likely to reduce light quantity and quality available to shorter species and to prevent seedling establishment. The great biomass of below-ground clonal organs recorded in this rhizomatous species suggests an important investment in resource storage (Suzuki & Stuefer 1999). This capacity could allow tiller production despite competition for light as stored resources can be mobilized to sustain the development of immature tillers (Stuefer & Huber 1999). Enhanced clonal multiplication together with low seedling establishment could explain the competitive success of *E. repens* in the absence of grazing. By contrast, grazing-induced defoliation impacts neighbor plants at a similar height and is consequently likely to remove more biomass from taller plants (Rotundo & Aguiar 2008). Similarly, the observed decrease in abundances of the late-seral dominant *Schizachyrium scoparium* in response to increasing grazing pressure seemed to be due to its inability to avoid grazing, despite its high tolerance to defoliation (Brown & Stuth 1993, Anderson & Briske 1995). Consequently and, contrary to our hypothesis, grazing response in the study species seemed to depend more on the balance

between competitive and avoidance abilities than on tolerance to defoliation (Noy-Meir *et al.* 1989).

A weak implication of clonal traits in species response to grazing

Only one clonal trait was associated to species response to grazing: the biomass of below-ground organs was negatively related to grazing resistance. As suggested above, this trait could be associated to competitiveness.

None other clonal trait was a good indicator of grazing resistance. Although species the most resistant to grazing tended to be tussock-forming and species the least resistant were rather rhizomatous or stoloniferous (Fig. 1, Table 2), the distance of clonal spread was not involved in species response to grazing. In a similar way, neither tillering nor connection branching were consistent with grazing resistance. Yet, numerous meristems situated close to or slightly below the ground surface are expected to sustain tillering following the removal of above-ground biomass (Briske 1996, Klimešová & Klimeš 2003). In grasses, the basal position of intercalary meristems, which protects them from above-ground damage, could be a key factor of tolerance to herbivory (Haukioja & Koricheva 2000). Indeed, plants of *S. scoparium* with a long grazing history produce significantly more, but smaller tillers than individuals from ungrazed populations (Briske & Anderson 1992). An increase in tiller density together with a decrease in tiller size in response to grazing have also been demonstrated in a clonal sedge (*Carex bigelowii*, Jónsdóttir 1991), confirming that grazing may stimulate tiller recruitment. Increases in tiller numbers have been recorded as a response to experimental defoliation in several grasses (Richards *et al.* 1988, Smith 1998). However, tillering responses to defoliation are very likely to depend on species (Richards *et al* 1988) or development stage of the plant at defoliation (Olson & Richards 1988, Bullock *et al* 1994). Such factors, notably the number and activity of buds available (structural blue-print *sensu* Huber *et al.* 1999), may have constrained the observed responses of ramet number to defoliation in the study species, independently from their grazing resistance.

The main objective of our study was to disentangle the relative involvement of species-specific trait values and trait response to defoliation in species resistance to grazing, with particular focus on clonal traits. Our results showed that only two traits related to the balance competition *vs.* avoidance could be used a predictors of species status in the studied system. In particular, species response to grazing was constrained neither by species clonal traits, nor by response of clonal traits to defoliation. Clonal traits and clonal responses to defoliation varied among species, inconsistently to species status in the field. Rather than a

species-based approach, a trait-based method might be more powerful in determining trait values favored by grazing.

Perspectives: from a species-based to a trait-based approach

Grazing, as other environmental factors, is expected to favor traits providing plants with resistance ability. This can be achieved through several ways: (i) the selection of species provided with these trait values, (ii) the intra-specific genetic variation of trait values and local adaptations or (iii) phenotypic plasticity and carry-over effects. Local adaptations refer to the selection of locally specialized genotypes of a species. The contrasted responses of individuals of *S. scoparium* (Briske & Anderson 1992) or *Bouteloua curpitendula* (Smith 1998) from populations with different grazing histories demonstrated that grazing may generate local genetic differentiation. However, our experimental design did not allow us to test for such local adaptations, notably because we collected the studied plants in a meadow where grazing pressure is not controlled. Phenotypic plasticity corresponds to the environment-mediated expression of a genotype (Bradshaw 1965). Contrary to our expectations of a positive relationship between grazing resistance and defoliation tolerance, plasticity in response to defoliation may enable species to occur in similar abundances whatever the grazing regime. Further studies of the expression of plant functional traits (in particular clonal traits) under contrasting regimes and associated defoliation would allow to determine the importance of trait values in response to grazing, and even to separate the ways by which their expression is favored (species selection, local adaptations or phenotypic plasticity).

Literature cited

Anderson VJ & Briske DD (1995) Herbivore-induced species replacement in grasslands: is it driven by herbivory tolerance or avoidance? *Ecological Applications*, 5: 1014-1024.

Belsky AJ (1987) The effects of grazing: confounding of ecosystem, community, and organism scale. *American Naturalist*, 129: 777-783.

Bradshaw AD (1965) Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics*, 13: 115-155.

Briske DD (1996) Strategies of plant survival in grazed systems: a functional interpretation. In: Hodgson J & Illius AW (eds) *The Ecology and management of grazing systems*. CAB International, Wallingford, pp 33-67.

Briske DD & Anderson VJ (1992) Competitive ability of the bunchgrass *Schizchyrium scoparium* as affected by grazing history and defoliation. *Vegetatio*, 103: 41-49.

Briske DD & Silvertown JW (1993) Plant demography and grassland community balance: the contribution of population regulation mechanisms. In: Baker MJ (ed) *Proceedings of the XVII International Grassland Congress*. SIR Publishing, Wellington, pp 291-298.

Brown JR & Stuth JW (1993) How herbivory affects grazing tolerant and sensitive grasses in a central Texas grassland: integrating plant response across hierarchical levels. *Oikos*, 67: 291-298.

Bullock MJ, Clear-Hill B & Silvertown J (1994) Tiller dynamics of two grasses - responses to grazing, density and weather. *Journal of Ecology*, 82: 331-340.

Bullock JM, Franklin J, Stevenson MJ, Silvertown J, Coulson SJ, Gregory SJ & Softs R (2001) A plant trait analysis of responses to grazing in a long-term experiment. *Journal of Applied Ecology*, 38: 253-267.

de Bello F, Leps J & Sebastià MT (2005) Predictive value of plant traits to grazing along a climatic gradient in the Mediterranean. *Journal of Applied Ecology*, 42: 824-833.

del-Val E & Crawley MJ (2004) Importance of tolerance to herbivory for plant survival in a British grassland. *Journal of Vegetation Science*, 15: 357-364.

del-Val E & Crawley MJ (2005) Are grazing increaser species better tolerators than decreasers? An experimental assessment of defoliation tolerance in eight British grassland species. *Journal of Ecology*, 93: 1005-1016.

Diaz S, Cabido M & Casanoves F (1998) Plant functional traits and environmental filters at a regional scale. *Journal of Vegetation Science*, 9: 113-122.

Diaz S, Lavorel S, McIntyre S, Falczuk V, Casanoves F, Michunas DG, Skarpe C, Rusch G, Sternberg M, Noy-Meir I, Landsberg J, Zhang W, Clark H & Campbell BD (2007) Plant trait responses to grazing – a global synthesis. *Global Change Biology*, 13: 313-341.

Diaz S, Noy-Meir I & Cabido M (2001) Can grazing response of herbaceous plants be predicted from simple vegetative traits? *Journal of Applied Ecology*, 38: 497-508.

Dykserhuis EJ (1949) Condition and management of rangelands based on quantitative ecology. *Journal of Range Management*, 2: 104-115.

Grime JP (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist*, 111: 1169-1194.

Halassy M, Campetella G, Canullo R & Mucina L (2005) Patterns of functional clonal traits and clonal growth modes in contrasting grasslands in the central Apennines, Italy. *Journal of Vegetation Science*, 16: 29-36.

Harper JL (1977) *Population Biology of Plants*. Academic Press, London.

Haukioja E & Koricheva J (2000) Tolerance in woody *vs.* herbaceous plants. *Evolutionary Ecology*, 14: 551-562.

Huber H, Lukacs S, Watson MA (1999) Spatial structure of stoloniferous herbs: an interplay between structural blue-print, ontogeny and phenotypic plasticity. *Plant Ecology*, 141:107-115.

Huntly N (1991) Herbivores and the dynamic of communities and ecosystems. *Annual Review of Ecology and Systematics*, 22: 477-503.

Jónsdóttir IS (1991) Effects of grazing on tiller size and population dynamics in a clonal sedge (*Carex bigelowii*). *Oikos*, 62: 177-188.

Keddy PA (1992) Assembly and response rules: two goals for predictive community ecology. *Journal of Vegetation Science*, 3: 157-164.

Klimeš L, Klimešovà J, Hendricks R & van Groenendael J (1997) Clonal plant architecture: a comparative analysis of form and function. In: de Kroon H & van Groenendael J (eds) *The ecology and evolution of clonal plants*. Backhuys Publishers, Leiden, pp 1-29.

Klimešovà J & de Bello F (2009) CLO-PLA: the database of clonal and bud bank traits of Central European flora. *Journal of Vegetation Science*, 20: 511-516.

Klimešová J & Klimeš L (2003) Resprouting of herbs in disturbed habitats: is it adequately described by Bellingham - Sparrow's model? *Oikos*, 103: 225-229.

Klimešovà J & Klimeš L (2008) Clonal growth diversity and bud banks of plants in the Czech flora: an evaluation using the CLO-PLA3 database. *Preslia*, 80: 255-275.

Kohler F, Gillet F, Gobat JM & Buttler A (2004) Seasonal vegetation changes in mountain pastures due to simulated effects of cattle grazing. *Journal of Vegetation Science*, 15: 143-150.

Lavorel S & Garnier E (2002) Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional Ecology*, 16: 545-556.

Lavorel S, McIntyre S. Landsberg J & Forbes TDA (1997) Plant functional classifications: from general groups to specific groups based on response to disturbance. *Trends in Ecology and Evolution*, 12: 474-478.

McNaughton SJ (1979) Grazing as an optimization process: grass-ungulate relationships in the Serengeti. *American Naturalist*, 113: 691-703.

Ménard C, Ducan P, Fleurance G, George JY & Lila M (2002) Comparative foraging and nutrition in horses and cattle in Europe wetlands. *Journal of Applied Ecology*, 38: 120-133.

Milchunas DG, Sala OE & Lauenroth WK (1988) A generalized model of the effects of grazing by large herbivores on grassland community structure. *American Naturalist*, 132: 87-106.

Noy-Meir I, Gutman M & Kaplan Y (1989) Responses of Mediterranean grassland plants to grazing and protection. *Journal of Ecology*, 77: 290-310.

Olson BE & Richards JH (1988) Tussock regrowth after grazing: intercalary meristems and axillary bud activity of tillers of *Agropyron desertorum*. *Oikos*, 51: 374-382.

Pakeman RJ (2004) Consistency of plant species and trait responses to grazing along a productivity gradient: a multi-site analysis. *Journal of Ecology*, 92: 893-905.

R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Richards JH (1993) Physiology of plants recovering from defoliation. In: Baker MJ (ed) *Proceedings of the XVII International Grassland Congress*. SIR Publishing, Wellington, pp 85-94.

Richards JH, Mueller RJ & Mott JJ (1988) Tillering in tussock grasses in relation to defoliation and apical bud removal. *Annals of Botany*, 62: 173-179.

Rossignol N, Bonis A & Bouzillé JB (2006) Relationships between grazing pattern and vegetation structure on the spatial variations of net N mineralisation in a wet grassland. *Applied Soil Ecology*, 31: 62-72.

Rotundo JL & Aguiar MR (2008) Herbivory resistance traits in populations of *Poa ligularis* subjected to historically different sheep grazing pressure in Patagonia. *Plant Ecology*, 194: 121-133.

Sammul M, Kull K, Niitla T & Möls T (2004) A comparison of plant communities on the basis of their clonal growth patterns. *Evolutionary Ecology*, 18: 443-467.

Schwaegerle KE, McIntyre H & Swingley C (2000) Quantitative genetics and the persistence of environmental effects in clonally propagated organisms. *Evolution*, 54: 452-461.

Smith SE (1998) Variation in response to defoliation between populations of *Bouteloua curtipendula* var. *caespitosa* (Poaceae) with different livestock grazing histories. *American Journal of Botany*, 85: 1266-1272.

Stowe KA, Marquis RJ, Hochwender CG & Simms EL (2000) The evolutionary ecology of tolerance to consumer damage. *Annual Review of Ecology and Systematics*, 31: 565-595.

Stuefer JF & Huber H (1999) The role of stolon internodes for ramet survival after clone fragmentation in *Potentilla anserina*. *Ecology Letters*, 2: 135-139.

Sultan SE & Spencer HG (2002) Metapopulation structure favors plasticity over local adaptation. *American Naturalist*, 160: 271-283.

Suzuki JI & Stuefer J (1999) On the ecological and evolutionary significance of storage in clonal plants. *Plant Species Biology*, 14: 11-17.

Tamm A, Kull K & Sammul M (2002) Classifying clonal growth forms based on vegetative mobility and ramet longevity: a whole community analysis. *Evolutionary Ecology*, 15: 383-401.

ter Braak CJF & Šmilauer P (1998) *CANOCO Reference Manual and User's Guide to Canoco for Windows: Software for Canonical Community Ordination (version 4).* Micro Computer Power, New York, Ithaca

Vesk PA & Westoby M (2001) Predicting plant species' responses to grazing. *Journal of Applied Ecology*, 38: 897-909.

Weiher E, van der Werf A, Thompson K, Roderick M, Garnier E & Eriksson O (1999) Challenging Theophrastus: a common core list of plant traits for functional ecology. *Journal of Vegetation Science*, 10: 609-620.

Conclusion du chapitre 1

Au cours de ce chapitre, nous avons mis en évidence l'impact du pâturage sur les traits clonaux. Cependant, un effet hiérarchique de l'inondation et du pâturage ont été mis en évidence. Le régime d'inondation constitue un filtre primaire, discriminant des communautés végétales de compositions clonales contrastées. La réponse des traits clonaux au pâturage est secondairement contrainte par le régime d'inondation et, de ce fait, diffère entre les communautés. Dans la communauté mésophile, la moins contrainte par le régime hydrique, la structure verticale (hauteur) de la végétation présente une hétérogénéité fine. Celle-ci n'est pas liée au régime de pâturage mais plutôt à des facteurs intrinsèques à la végétation (*e.g.* composition spécifique) et/ou d'autres facteurs environnementaux (*e.g.* caractéristiques du sol). La défoliation générée sous pâturage intense homogénéise la hauteur de la végétation, tandis qu'en pâturage modéré, la défoliation augmente l'hétérogénéité mais à large échelle (supérieure à 1 mètre). Par conséquent, quel que soit le régime de pâturage, la probabilité que la défoliation soit perçue de manière homogène par le fragment clonal est forte. A l'échelle du fragment clonal, la défoliation est susceptible de ne varier que par sa fréquence d'occurrence ou son intensité.

Le pâturage quel que soit son régime, tend à favoriser les formes clonales stolonifères ou cespiteuses, les banques de bourgeons végétatifs aériennes, les ramets annuels et les connexions à courte durée de vie. Des organes et des bourgeons clonaux souterrains, des connexions et des durées d'intégration longues ainsi que des taux de multiplication clonale forts sont, quant à eux, caractéristiques des conditions non pâturées.

A l'issue de ces observations, il semble donc que le pâturage favorise les formes clonales stolonifères et cespiteuses. L'architecture clonale, quant à elle, semble peu associée à la résistance au pâturage, tandis que la capacité de mise en place de réserves (estimée par la biomasse des organes clonaux souterrains) et de leur réallocation en réponse à la défoliation serait une caractéristique des espèces les moins résistantes au pâturage. En outre, la réponse des traits clonaux à la défoliation ne semble pas liée à la résistance des plantes au pâturage.

Bien que ce premier chapitre nous ait donné un premier aperçu des traits clonaux potentiellement impliqués dans la réponse au pâturage, des expérimentations semblent nécessaires pour comprendre l'implication de propriétés clonales majeures, l'architecture clonale et le stockage de ressources, dans la réponse à la défoliation et au pâturage. Dans un

premier temps, nous avons donc cherché à caractériser la réponse morphologique et architecturale à la défoliation chez plusieurs espèces clonales (CHAPITRE 2). Dans un second temps, nous avons étudié l'implication potentielle de la capacité de stockage dans la tolérance à la défoliation et dans la réponse au pâturage (CHAPITRE 3).

CHAPITRE 2 – REPONSES MORPHOLOGIQUES ET ARCHITECTURALES DES PLANTES CLONALES A LA DEFOLIATION.

Introduction du chapitre 2

La croissance clonale confère aux plantes la capacité de s'étendre horizontalement par le biais de connexions pouvant être souterraines (rhizomes) ou aériennes (stolons). L'architecture clonale, c'est-à-dire la structure et la forme du réseau de connexions, est une propriété cruciale, contraignant la disposition des ramets. Elle est décrite par les propriétés d'élongation et de ramification des connexions (nombre et longueur des connexions primaires et des ramifications, distances inter-ramets, angles entre les connexions, etc.). La disposition spatiale des ramets détermine à la fois la capacité d'une plante clonale à coloniser et à occuper l'espace disponible, mais également l'intensité de la compétition intra-ramets. L'architecture exprimée par le fragment clonal dépend de plusieurs facteurs.

L'architecture clonale peut être limitée par des contraintes structurales spécifiques (*structural blue-print*) telles que le nombre de bourgeons végétatifs disponibles. Elle peut également être modulée par les conditions environnementales. Ainsi, plusieurs études ont démontré cette plasticité phénotypique architecturale en réponse aux ressources édaphiques ou lumineuses. Cependant, les ajustements plastiques de l'architecture clonale sont parfois limités ou inexistants. En effet, le type de connexions (rhizomes ou stolons) ou les contraintes structurales sont des facteurs pouvant limiter la plasticité architecturale. Par exemple, les stolons seraient des connexions plus plastiques que les rhizomes.

Chez des plantes non clonales, la défoliation modifie les relations hiérarchiques entre les bourgeons, notamment en provoquant la levée de la dominance apicale. Les modifications architecturales des structures aériennes qui en résultent sont souvent évoquées comme mécanismes de tolérance à la défoliation. Par exemple, la régénération végétative causée par l'activation de bourgeons végétatifs dormants, principalement étudiée chez les plantes ligneuses, serait un mécanisme de tolérance aux perturbations.

L'objectif de ce chapitre est donc d'identifier et de caractériser la plasticité de l'architecture clonale en réponse à la défoliation des ramets. Les hypothèses suivantes ont été testées :

1- La plasticité de l'architecture clonale permet le maintien de la performance clonale en réponse à la défoliation des ramets (ARTICLES 4 ET 5).

2- L'architecture clonale et sa plasticité en réponse à la défoliation dépendent de contraintes constitutives telles que :

a. le type de connexions produites. Nous supposons que les stolons sont plus plastiques que les rhizomes (ARTICLE 4)
b. les contraintes structurelles, *i.e. structural blue-print* (ARTICLE 5).

Une expérience en serre, sur dix espèces clonales (cinq stolonifères et cinq rhizomateuses) ainsi qu'une expérience en jardin sur deux espèces Cyperaceae rhizomateuses ont été mises en place pour tester ces hypothèses.

Article 4 – Responses of clonal architecture to experimental defoliation: a comparative study between ten grassland species.

Marie-Lise Benot[1], Cendrine Mony[1], Sara Puijalon[2], Majid Mohammad-Esmaeili[1-3], Jacques J. M. van Alphen[1], Jan-Bernard Bouzillé[1] & Anne Bonis[1]

[1]UMR CNRS 6553, ECOBIO, Université de Rennes 1, Campus de Beaulieu, 35042 Rennes Cedex, France

[2]UMR CNRS 5023, Ecology of Fluvial Hydrosystems, Université Lyon 1, 69622 Villeurbanne Cedex, France

[3]Faculty of Rangeland and Watershed, Gorgan University of Agricultural Sciences and Natural Resources, Shahid Beheshti Street, Gorgan, Iran

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Abstract

Clonal architecture may enable plants to effectively respond to environmental constraints but its role in plant tolerance to defoliation remains poorly documented. In several non-clonal species, modifications of plant architecture have been reported as a mechanism of plant tolerance to defoliation, yet this has been little studied in clonal plants.

In a glasshouse experiment, five rhizomatous and five stoloniferous species of grazed pastures were subjected to three frequencies of defoliation in order to test two hypotheses. (1) We expected plant clonal response to defoliation to be either a more compact architecture (low clonal propagation, but high branching), or a more dispersed one (long-distance propagation and low branching). Such plastic adjustments of clonal architecture were assumed to be involved in tolerance to defoliation *i.e.* to promote genet performance in terms of biomass and number of ramets. (2) The response of clonal architecture to defoliation was expected to be dependent on the species and to be more plastic in stoloniferous than in rhizomatous species.

Most genets of each species were tolerant to defoliation as they survived and developed in every treatment. Architectural modifications in response to defoliation did not match our predictions. Clonal growth was either maintained or reduced under defoliation. Relative growth rate (RGR) decreased in eight species, whereas defoliated genets of seven species produced as many ramets as control genets. Biomass allocation to ramet shoots remained stable for all but one species. In defoliated genets, the number and mean length of connections, and mean inter-ramet distance were equal or lower than in control genets. Four groups of species were distinguished according to their architectural response to defoliation and did not depend on the type of connections. We hypothesised that dense clonal architectures with low plasticity may be the most advantageous response in defoliated conditions such as in grazed pastures.

Keywords

Between-class PCA; glasshouse experiment; response groups; short-term defoliation; tolerance.

Introduction

Vegetative multiplication is widely spread in plants, particularly in Angiosperms. Many species are able to reproduce both by seeds (sexual reproduction) and by clonal growth (asexual reproduction), while some others are even exclusively clonal (Price & Marshall 1999). A genet consists of the vegetative production of genetically identical offsprings (*ramets*) that are potentially independent units as they develop their own shoots and roots (Hutchings & Bradbury 1986; Klimeš *et al*. 1997). This propagation allows the genet to persist and spread both in space and time (Gardner & Mangel 1997; Oborny & Kun 2002). Vegetative growth modes are variable and major attention has been paid to clonal plants forming aboveground or belowground horizontal stems (stolons and rhizomes respectively, hereafter often referred as *connections*) carrying ramets (Klimeš *et al*. 1997). In the following, the term *clonality* will refer to these particular growth modes.

Clonal architecture provides singular plant characteristics relying mainly on the integration between ramets, which presents a potential adaptive role (Hutchings & Wijesinghe 1997; Suzuki & Stuefer 1999). Clonal plants are likely to effectively respond to environmental constraints that may explain their abundance in a variety of environments (Hutchings 1999; Price & Marshall 1999). Clonal plants are particularly able to show plastic adjustments of clonal architecture, a strategy involved in selective foraging for light quality (Stuefer & Huber 1998), light intensity (Dong & Pierdominici 1995), nutrient availability (Liao *et al*. 2003) and even soil temperature (MacDonald & Lieffers 1993) and competition (MacDonald & Lieffers 1993; Kleijn & van Groenendael 1999; Macek & Lepš 2003).

For plants submitted to defoliation, the ability of growth and reproduction after damage is defined as tolerance, while the term compensation is often used to characterise the degree of this tolerance (Strauss & Agrawal 1999). Tolerance can be considered as a plastic trait and ranged along a gradient (Maschinski & Whitham 1989, Stowe *et al*. 2000) Incomplete tolerance occurs when defoliated plants survive and develop but their performance is lower than for undefoliated plants (undercompensation *sensu* Strauss & Agrawal 1999). Compensating and even overcompensating tolerance respectively refer to maintained and increased performance for damaged plants compared to undamaged ones (Stowe *et al*. 2000). Amongst a variety of mechanisms, modifications of aboveground plant architecture (*i.e.* branching pattern) had often been mentioned as a frequent response to clipping in non-clonal plants (Owen 1980; Paige & Whitham 1987; Lennartsson *et al*. 1998).

The aim of this study was to investigate whether active adjustments of clonal architecture are involved in genet tolerance to defoliation. Few studies have been carried out

on the responses of clonal architecture to disturbance such as clipping or grazing (see however Moen *et al*. 1999; Piqueras 1999; Li *et al*. 2004; Wang *et al*. 2004). We investigated such questions by considering the comparative response to experimental clipping for ten species, embracing both stoloniferous and rhizomatous species.

We first tested whether clonal architecture-related traits are involved in species compensating tolerance of defoliation, enabling the maintenance of genet performance (in terms of biomass and ramet production). Their response is expected to vary between traits, and either a more compact architecture (low clonal propagation, but high branching) or a more dispersed one (long-distance propagation and low branching) are expected to occur in response to defoliation (Table 1). The second hypothesis was that the response of clonal architecture to defoliation might vary according to species and in particular among stoloniferous and rhizomatous species. As stolons are more often involved in spatial propagation and show higher morphological plasticity than rhizomes (Dong & de Kroon 1994; Dong & Pierdominici 1995), clonal architecture is expected to be more responsive to defoliation in stoloniferous than in rhizomatous species.

Table 1. Variations of clonal traits predicted by the hypothesis of compensating tolerance to defoliation. Arrows indicate the direction (decrease, maintenance or increase) of trait variation between undefoliated and defoliated plants.

Survival and development		→	
Clonal performance			
Relative Growth Rate		→	
Number of ramets		→	
Biomass allocation to ramets		→	
Clonal architecture-related traits			
Number of connections	↘		↗
Mean length of connections	↗		↘
Mean inter-ramet distance	↗		↘
Clonal growth form	Dispersed		Compact

Methods

The response of ten clonal species to three frequencies of defoliation was recorded in terms of genet performance and architecture-related traits.

The studied plant species were selected out of the twenty-three clonal herbaceous perennials from grazed pastures in the Marais poitevin, Western France (46°28′N and 1°30′W). These species are the most abundant clonal species in these plant communities, where the biomass consumption by grazing ranges from 55% to 87% of the available biomass (Rossignol *et al*. submitted). They belong to several families and show different modes of clonal growth. All species can produce long connections, either aboveground (stolons) or belowground (rhizomes), or both. Some of them can also form tussocks through very short connections (caespitose growth form) (Table 2).

Table 2. Studies species and their clonal growth type (adapted from Klimeš *et al*., 1997). For species having the ability to develop two types of connections, the major type developed during the experiment is mentioned first. Caespitose growth type corresponds to the production of short rhizomes (tussock forming species).

Species	Abbreviation	Class	Family	Clonal growth type
Agrostis stolonifera L.	Asto	Monocotyledons	Poaceae	Stoloniferous Caespitose
Carex divisa Huds.	Cdiv	Monocotyledons	Cyperaceae	Rhizomatous
Eleocharis palustris Rœm. & Schult.	Epal	Monocotyledons	Cyperaceae	Rhizomatous
Elytrigia repens L.	Erep	Monocotyledons	Poaceae	Caespitose Rhizomatous
Glyceria fluitans (L.) R. Br.	Gflu	Monocotyledons	Poaceae	Caespitose Stoloniferous
Juncus articulatus L.	Jart	Monocotyledons	Juncaceae	Rhizomatous Stoloniferous
Juncus gerardii Lois.	Jger	Monocotyledons	Juncaceae	Rhizomatous
Ranunculus repens L.	Rrep	Dicotyledons	Ranunculaceae	Stoloniferous
Trifolium fragiferum L.	Tfra	Dicotyledons	Fabaceae	Stoloniferous
Trifolium repens L.	Trep	Dicotyledons	Fabaceae	Stoloniferous

Experimental set-up

Ramets were collected in February 2006, from a grazed area of 1 hectare. Thirty-three ramets per species were chosen and randomly assigned to one of the three treatments of defoliation. The experimental design thus consisted of 10 species × 3 defoliation treatments × 11 replicates with a total of 330 experimental units.

Each ramet was cleaned, weighed (fresh mass, FM) and rooted in the centre of a pot (20-cm-diameter and 16-cm-height) containing fine garden soil. Six to 10 cm^3 of substrate from the collection site were added close to the roots of the ramet in order to provide symbiotic microorganisms. Ramets were first allowed to grow freely for a five-week acclimation period during which dead ramets were replaced. The ratio FM/DM (fresh and dry mass respectively) was determined for ten additional non-planted ramets per species, and the mean per species used to estimate initial dry biomass of each planted ramet.

The experiment was conducted in a non-heated glasshouse at the campus of Beaulieu (University of Rennes 1, France) from 29th March up to 17th May 2006. In the glasshouse, temperature was maintained below 25°C. Pots were watered daily with tap water, and weeds were regularly removed.

Experimental treatments

The three frequencies of defoliation tested were: no defoliation (control treatment), defoliation every thirty days (mid-frequency defoliation treatment) and defoliation every fifteen days (high-frequency defoliation treatment). To make a realistic simulation of defoliation caused by cattle, all aboveground parts of Monocotyledons were cut to 7 cm height (Loucougaray *et al*. 2004). As Dicotyledons were generally lower than 7 cm, defoliation consisted in the removal of 50 % of developed leaves by severing the petiole at its base (both petiole and lamina were removed). Genets were harvested 8 weeks after the first clipping. Genets under mid-frequency treatment were thus cut twice and those under high-frequency treatment were cut four times.

Trait measurements

At harvest the number of ramets per genet was counted and architectural traits were measured. Then, each genet was divided into vegetative shoots, flowers, connections, and roots, dried to constant mass at 60°C and the dry mass of each of these parts was weighed. As the study focused on clonal architecture, biomass allocation to roots was not taken into account. As

only a few genets had produced flowers during the experiment, only traits related to vegetative development were taken into account.

Traits related to clonal performance

The investment in clonal reproduction was estimated as the final number of ramets. The total growth of each genet was measured as the relative growth rate calculated as follows: $RGR=\frac{\ln(final biomass+clippings)-\ln(initial biomass)}{number of days}$, where *final biomass* is the dry mass of the whole genet (including roots) at the end of the experiment, *clippings*, the dry mass of clipped tissues, and *initial biomass*, the dry mass of the planted ramet. Finally, the biomass of ramets corresponded to the sum of the final dry mass of shoots of all ramets produced by the genet.

Architecture-related traits

Measured traits were: the total number of connections (stolons or rhizomes) per genet, the mean length of connections (with a precision of 1 mm) produced per genet, and the mean inter-ramet distance (with a precision of 1 mm) per genet. This latter corresponded to the mean distance between two consecutive ramets. It could be calculated only for genets that had produced connections carrying ramets (Fig. 1).

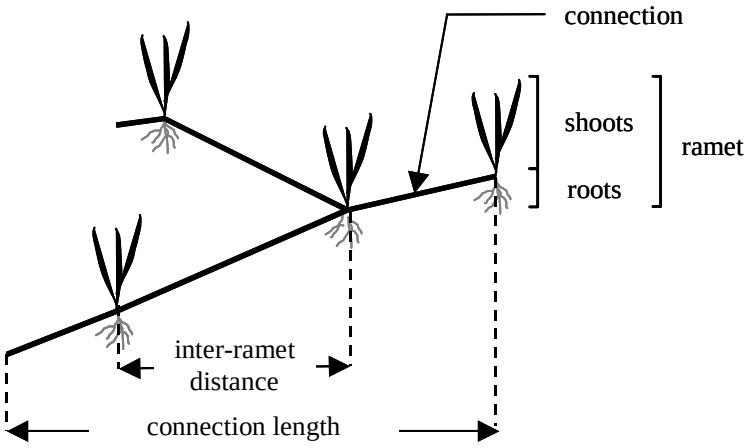

Fig. 1 Schematic representation of a genet, and definition of architectural traits.

Four out of ten species can develop two types of connections (Table 2). However, *Juncus articulatus* produced very few stolons and only data related to rhizomes were taken into account. For *Agrostis stolonifera*, *Elytrigia repens* and *Glyceria fluitans*, the calculation of mean inter-ramet distance did not include caespitose connections.

A species was considered as tolerant when genets had survived and developed even under the high-frequency defoliation treatment. The responses of clonal performance to defoliation (from a decrease to an increase) were used to characterise the degree of tolerance (from incomplete to compensating tolerance).

Statistical analysis

For all species, one-way ANOVAs showed no differences in ramet initial biomass between the three treatments, confirming the assumption of ramet randomisation between treatments at the beginning of the experiment. The percentage of biomass removed by defoliation was assessed through ANOVA with species and treatment as main effects. The correlation between the percentage of biomass removed and the values of architecture-related traits was tested. We used the non parametric Spearman correlation coefficient (ρ) as traits did not follow a normal distribution.

Within-species effects of defoliation treatments on final number of ramets, RGR, mean length of connections and mean inter-ramet distance were assessed through one-way ANOVAs, after checking for normality and homogeneity of variances (Kolmogorov-Smirnov and Levene tests respectively), and log-transformation of data when necessary. Post-hoc comparisons between treatments were tested by the Tukey-Kramer test. In the particular case of final number of connections, for which assumptions of normality and homogeneity of variances were not met, non-parametric Kruskal-Wallis tests were used and post-hoc comparisons were made by Mann and Whitney U tests with Bonferroni correction. The effect of defoliation on biomass allocation to ramets was analysed by ANCOVAs using final biomass as a covariate. The aim was to increase the power of the F-statistic by adjusting for the influence of the covariate, and to avoid the use of biomass ratios, which may be misleading to study allocation patterns (Jiaseński & Bazzaz 1999). Interactions between treatment and covariate were first introduced into the model and removed when non-significant.

The comparison of architectural responses to defoliation between the ten species was done by multivariate analyses, taking into account the three architecture-related traits. After a Principal Components Analysis (PCA) on correlation matrix, traits were centred independently per species (within-species PCA) and compared between treatments by a between-class PCA (bc-PCA), each treatment considered as one class (Dolédec & Chessel 1991). Such analysis enabled to eliminate scale effects linked to differences of average trait values between species. Following this analysis, hierarchical ascendant classification (HAC

using Ward method of clustering, Legendre & Legendre 1998) was used to group species according to their multivariate trajectory of response. The coordinates of each treatment along the two first axes of bc-PCA constituted the six variables.

ADE-4 software (Thioulouse *et al*. 1997) was used for bc-PCA, and JMP software (SAS procedure) for other statistical calculations. In all cases, we rejected null hypothesis at the p=0.05 level.

Results

Effects of defoliation treatments on clonal performance and architecture-related traits

The amount of biomass removed by defoliation differed significantly between species and treatments (species × treatment: $F_{18;284} = 12.05$, $P < 0.0001$, species: $F_{9;284} = 36.78$, $P < 0.0001$, treatment: $F_{2;284} = 750.04$, $P < 0.0001$). For the high-frequency defoliation treatment, it ranged from 42.5 % (*A. stolonifera*) to 10 % (*Juncus gerardii*). The three grasses were the most severely impacted (between 27 % and 42.5 % for high-frequency defoliation, and between 20 % and 24 % for mid-frequency treatment), whereas *J. gerardii* and the two *Trifolium* species were less impacted, especially by mid-frequency defoliation treatment (only from 5 % to 7 % biomass removed; Fig. 2).

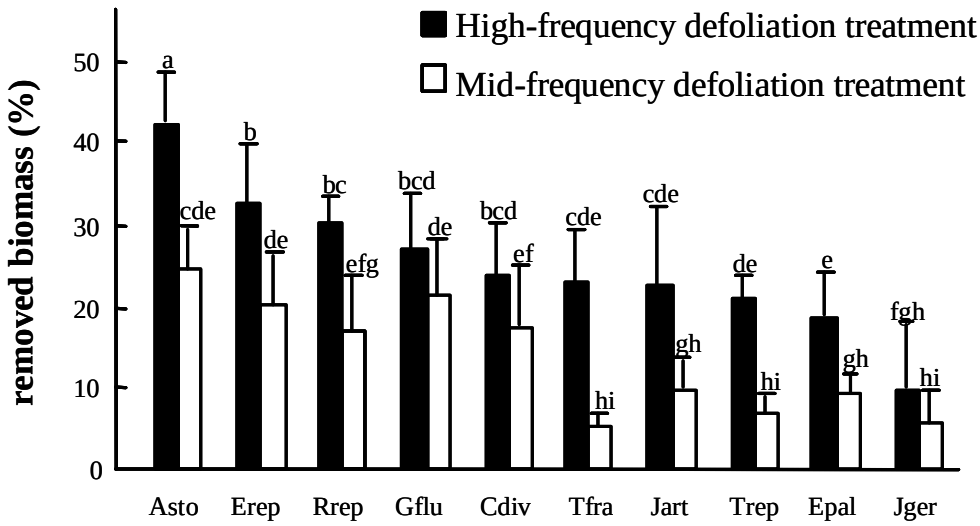

Fig. 2 Means and standard deviations of percentage of biomass removed [biomass removed/(final biomass + biomass removed)] for each species. Letters indicate significant differences between treatments and species (post-hoc Tukey tests).

At the end of the experiment, 311 out of the 330 genets had had survived and developed, all species and treatments taken together. The impact of treatments on trait values depended on species and traits. When significant, differences of trait values occurred either between the control and both defoliation treatments or between the control and the high-frequency defoliation treatment only. Therefore, hereafter the term defoliation will most frequently be used without distinction between the two levels of the defoliation treatment. *J. articulatus, J. gerardii* and *Ranunculus repens* were the three species for which almost no trait was significantly impacted by defoliation. By contrast, the final number of ramets of defoliated genets was 60 % to 30 % compared to the one of control genets for *Eleocharis palustris, G. fluitans* and *Trifolium fragiferum*. It did not significantly change for the other ones (Table 3; see also Table S1A in Supplementary Material). Defoliation generated a significant reduction of RGR for eight species (Table 3, Table S1B), and there was a significant covariation between the final biomass and the biomass of ramet shoots for all species except *T. fragiferum*. This last trait was significantly impacted by clipping treatment only for *Trifolium repens*, indicating that, for the nine other species, biomass allocation to shoots (*i.e.* the part of the genet final biomass allocated to the ramet shoots) was not changed by defoliation (Table 3, Table S1C). For *Carex divisa* only, all architecture-related traits showed a significant drop after defoliation (Table 3). For *E. palustris, E. repens* and *T.fragiferum*, the number of connections decreased, up to 80 % for *E. palustris* (Table 3, Table S2A). Mean length of connections decreased by 75 % for *A. stolonifera* and 50 % for *C. divisa* and *G. fluitans* (Table 3, Table S2B). Finally, mean inter-ramet distance decreased in *A. stolonifera, C. divisa* and *T. repens* (Table 3, Table S2C).

Multivariate responses of clonal architecture to defoliation

Over all ten species, there was no significant correlation between the percentage of biomass removed and the three architectural traits ($\rho = -0.08$, $P = 0.15$ for the number of connections; $\rho = 0.02$, $P = 0.68$ for the mean length of connections, and $\rho = 0.02$, $P = 0.68$ for the mean inter-ramet distance). But their response to defoliation varied between traits and species. The two first axes of bc-PCA represented 96 % of total variance (69 % and 27 % respectively, Fig. 3). The F1 axis carried out mean length of connections and mean inter-ramet distance. The F2 axis was negatively correlated with the number of connections (Fig. 3A). The amplitude of variation between the extremes of the trajectories along the first axis was weak for all species but *A. stolonifera*.

Table 3. Effects of defoliation on clonal traits for all ten species. Arrows indicate the variations of trait values between control and both defoliation treatments taken altogether: ↘ significant decrease, → no significant difference, NA not available. Abbreviations of species follow table 2. Results of statistical tests are presented in Supplementary Material (Table S1 for traits indicative of performance, Table S2 for architectural traits).

	First hypothesis		*Asto*	*Cdiv*	*Epal*	*Erep*	*Gflu*	*Jart*	*Jger*	*Rrep*	*Tfra*	*Trep*
Survival and development	→		→	→	→	→	→	→	→	→	→	→
Clonal performance												
RGR	→		↘	↘	↘	↘	↘	→	→	↘	↘	↘
Number of ramets	→		→	→	↘	→	↘	→	→	→	↘	→
Biomass allocation to ramets*	→		→	→	→	→	→	→	→	→	→	↘
Clonal architecture												
Number of connections	↗	↘	→	↘	↘	↘	→	→	→	→	↘	→
Mean length of connections	↘	↗	↘	↘	→	→	↘	→	→	→	→	→
Mean inter-ramet distance	↘	↗	↘	↘	→	NA	NA	→	→	→	→	↘

* Biomass allocation to ramets corresponds to the effect of treatment (main factor) on the biomass of all ramets of a genet, tested by the ANCOVA (final genet biomass as a covariate).

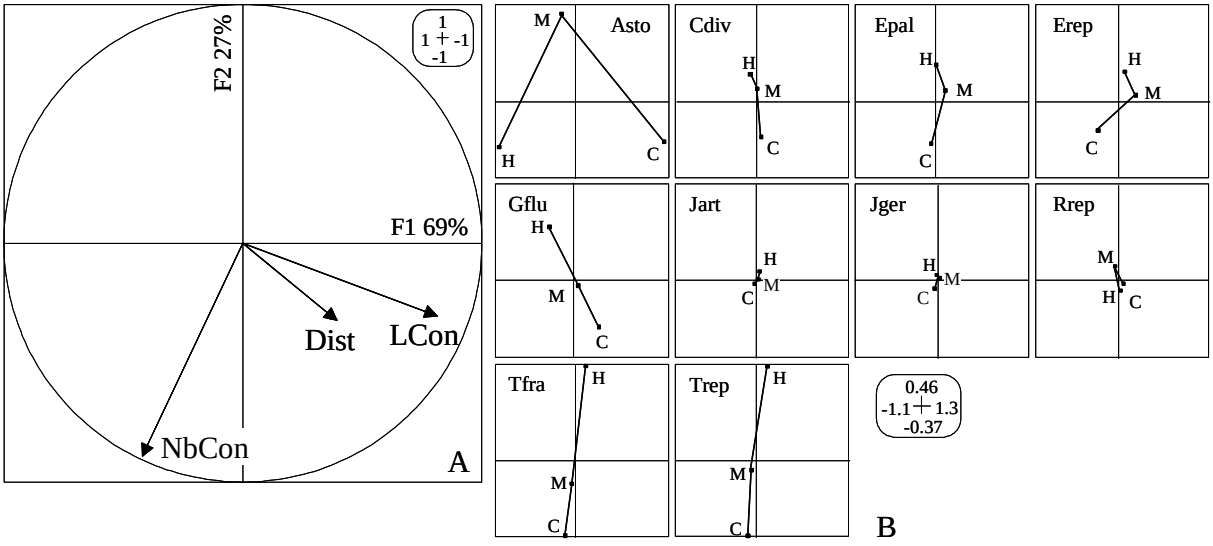

Fig. 3 Multivariate responses of clonal architecture to defoliation for each species. Traits values have been centred per species (within-species PCA) and compared between treatments (between-treatment PCA). The factorial plan is thus the same for all species. A. Correlation circle of architectural traits in the factorial plan 1-2 of between-treatment PCA. B. Trajectories of multivariate responses to the three treatments for each species in the factorial plan 1-2. Each point represents the barycentre of all replicates of a defoliation treatment. Points C: control treatment, M: mid-frequency defoliation treatment, H: high-frequency defoliation treatment.
Dist: mean inter-ramet distance, LCon: mean length of connections, NbCon: number of connections. See Table 2 for the meaning of species abbreviations.

The trajectory between control and high-frequency defoliation treatment along the second axis varied in the direction of a decreased number of connections for nine species. This variation was the most important for *Trifolium* species, weaker for *C. divisa, E. palustris, E. repens* and *G. fluitans*, and was close to zero for Juncaceae and *R. repens*. The trajectory of *A. stolonifera* along this axis had a singular shape, with a great increase between control and mid-frequency defoliation and a decrease between mid-frequency and high-frequency defoliation (Fig. 3B).

The HAC based on architectural responses to defoliation resulted in four groups of species. The first group consisted of both *Trifolium* species. *J. articulatus, J. gerardii* and *R. repens* formed the second group, while *C. divisa, E. palustris, E. repens* and *G. fluitans* constituted a third group. Finally, the last group corresponded to *A. stolonifera*, due to the singular shape of its trajectory of response (Fig. 4).

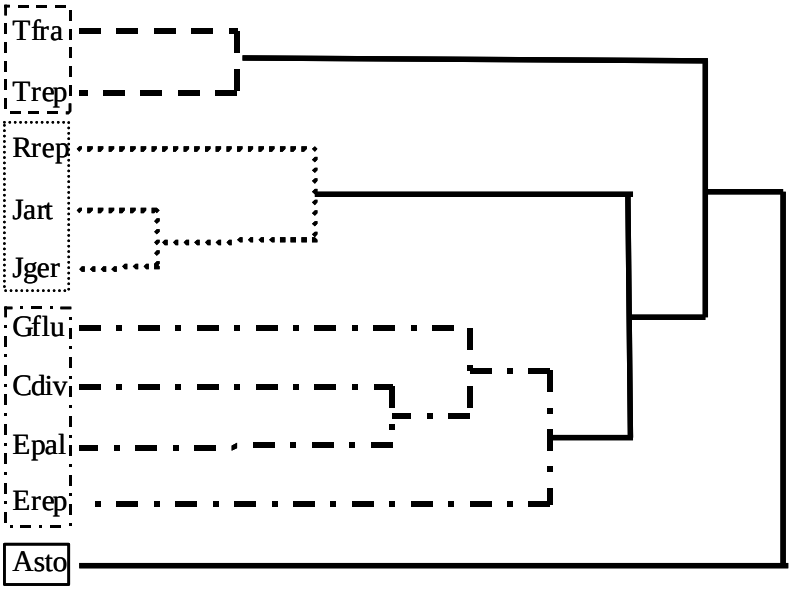

Fig. 4 Dendrogram of species resulting of HAC according to the multivariate responses of clonal architecture to defoliation. Species were clustered into four groups on the base of. Euclidean distance (Ward's method). See Table 2 for the meaning of species abbreviations.

Discussion

Consequences of defoliation on clonal performance

All species showed tolerance to defoliation as 311 out of 330 genets survived and developed even when severely clipped. Biomass allocation to ramets was not affected by the treatment, except for one species. This indicates that genets were able to sustain damaged ramets and even to compensate for aboveground biomass removal caused by defoliation. While the design did not allow to identify the mechanisms involved, Brown & Allen (1989) reported that clipping treatment may cause the translocation of resources from belowground organs to support the regrowth of aboveground tissues.

We recorded however a great discrepancy on the degree of tolerance depending on traits and species considered. The RGR decreased with clipping, which is typical of undercompensation (Strauss & Agrawal 1999) and incomplete tolerance (Stowe *et al*. 2000), while together, seven out of 10 species maintain a similar number of ramets in all three treatments, arguing for species compensating tolerance to clipping. Previous studies have already reported a variety of responses to clipping by clonal plants, among species and among environments for a given species. For example ramet number has been reported to decrease for three clonal perennials with leaf removal, (Hicks & Turkington 2000), to be unchanged for *Leymus chinensis* (Wang *et al*. 2004), either to be unchanged or to increase according to nutrient availability for *Cyperus esculentus* (Li *et al*. 2004) and even to increase for five caespitose grasses (Richards *et al*. 1988). Biomass responses to defoliation were also shown to vary from undercompensation (Li *et al*. 2004) to overcompensation (Hicks & Turkington 2000), probably due to both the differing species studied and the defoliation treatment applied. Interspecific differences in compensatory ability were notably found both in clonal and non-clonal species (McNaughton & Chapin 1985, Belsky 1986, Del-Val & Crawley 2005). Response to defoliation has also been shown to vary within the same species depending on environmental conditions (Maschinski & Whitham 1989). The lack of generality in clonal plant responses to clipping may also originate from the variety of defoliation treatments used in the different studies. Their impact on plant growth may indeed differ whether they are applied at a given date (*e.g.* Hicks & Turkington 2000, Wang *et al*. 2004), at a given development stage (*e.g.* Richards *et al*. 1988), or several times (*e.g.* Li *et al*. 2004).

Responses of clonal architecture-related traits to defoliation

Clonal architecture-related traits did not match the predicted responses. Species followed four types of architectural response to defoliation, going from no to high variation**.** Trait values never increased with clipping and defoliation thus led to fewer connections and/or shorter connections and mean inter-ramet distances. Similar results in response to clipping or grazing have already been observed in other herbaceous clonal plants such as *Trifolium repens* (Hay & Newton 1996), *Acaena magellanica* (Moen *et al*. 1999), *Trientalis europaea* (Piqueras 1999) or *Lymus chinensis* (Wang *et al*. 2004).

Furthermore, the results obtained here show that there was no relationship between the multivariate pattern of architectural response and the degree of tolerance to defoliation. For instance, both the first and the third response groups included together species for which defoliation induced no change in the number of ramets (*C. divisa*, *E. repens* and *T. repens*), and species for which defoliation induced a decreased number of ramets (*E. palustris*, *G. fluitans* and *T. fragiferum*). Compensating tolerance can be related to various responses of architectural traits. For *E. repens*, the production of ramets was not affected by defoliation despite a decrease of the number of connections, as only a few ramets were produced by these connections, the majority being caespitose (tussock forming). An alternative strategy was shown by *C. divisa*, *T. repens* and *A. stolonifera*. They maintained the number of ramets unchanged with clipping by the way of the decreased mean inter-ramet distance, whatever the variation of the other traits.

Interspecific comparison of the responses of clonal architecture to defoliation

Previous studies have shown that higher nutrient and/or light supply increased branching intensities of both stolons and rhizomes, whereas the morphology of stolons (mean connection length and mean internode length) was more plastic than the morphology of rhizomes (Dong & de Kroon 1994; Dong & Pierdominici 1995). According to our study, only the stolons of *A.stolonifera* showed a high degree of variability, with high amplitude of variation of all architecture related-traits. The responses of other stoloniferous species were not clearly distinct from those of rhizomatous species. The second and the third response groups contained both stoloniferous (*G. fluitans* and *R. repens*) and rhizomatous species (*C. divisa*, *E. palustris*, *E. repens*, *J. articulatus* and *J. gerardii*). Thus, contrary to the predictions that clonal architecture should be more responsive to defoliation in stoloniferous than in rhizomatous species, the response of clonal architecture to defoliation was not constrained by the type of connections. Other developmental constraints may be implied in architectural

responses to defoliation. For instance, branching pattern is related to the number and activity of axillary meristems (Huber & During 2001), which may play a key role in architectural response to defoliation (Briske 1996). In monopodial species (as *T. fragiferum* and *T. repens*) the number of connections is constrained by the limited number of meristems available per ramet (Huber & During 2001). As a result they are likely to be more sensitive to defoliation (Huber *et al*. 1999). Indeed, the trajectories of response of the two studied *Trifolium* species did show a great decrease of the number of connections, compared to the eight others species (sympodial species, Klimeš & Klimešova 1999).

However, the species constitutive of the second (*J. articulatus*, *J. gerardii* and *R. repens*) and the third (*C. divisa*, *E. palustris*, *E. repens* and *G. fluitans*) response groups can be linked neither by their phylogenetic nor by their developmental features. Our results thus demonstrate that architectural response to defoliation cannot be predict on the sole basis of the type of clonal connection (stolons or rhizomes), nor by the phylogenetic and developmental features.

Conclusion

Species responses to defoliation were either the stability of clonal architecture or the decreased investment in the production of connections and a lower clonal propagation. Gross *et al*. (2007) showed that low lateral spread was a constitutive trait of species tolerant to clipping. Such growth forms can be interpreted as the expression of the '*consolidation strategy*' (as defined by de Kroon & Schieving 1990) characterised by short and little plastic connections. In grazed pastures, where the risk of defoliation is high, plants with short propagation (either constitutive or induced by defoliation), capable of producing a dense population of ramets when defoliated are very likely to be more competitive than plants that allocate energy in the production of long connections. However, small variations of architectural traits may have great consequences on spatial distribution of ramets within a genet, and consequently on genet performance, resource acquisition and competition (Huber *et al*. 1999). Such parameters are likely to be modified by recurrent defoliation that occurs in grazed pastures. The four architectural patterns of response to defoliation distinguished during the present short-term experiment are very likely to constrain competitive ability and hence the relative species abundances along the grazing gradient.

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Literature cited

Belsky AJ (1986) Does herbivory benefit plants? A review of the evidence. *American Naturalist*, 127: 870-892.

Briske DD (1996) Strategies of plant survival in grazed systems: a functional interpretation. In: Hodgson J & Illius AW (eds) *The Ecology and management of grazing systems*. CAB International, Wallingford, pp 33-67.

Brown B & Allen T (1989) The importance of scale in evaluating herbivory impacts. *Oikos*, 54: 189-194.

de Kroon H & Schieving F (1990) Resource partitioning in relation to clonal growth strategy. In: van Groenendael J & de Kroon H (eds) *Clonal growth in plants: regulation and function*. SPB Academic Publishing, The Hague, pp 113-130.

Del-Val E & Crawley MJ (2005) Are grazing increaser species better tolerators than decreasers? An experimental assessment of defoliation tolerance in eight British grassland species. *Journal of Ecology*, 93: 1005-1016.

Dolédec S & Chessel D (1991) Recent development in linear ordination methods for environmental sciences. *Advances in Ecology*, 1: 133-155.

Dong M & de Kroon H (1994) Plasticity in morphology and biomass allocation in *Cynodon dactylon*, a grass species forming stolons and rhizomes. *Oikos*, 70: 99-106.

Dong M & Pierdominici MJ (1995) Morphology and growth of stolons and rhizomes in three clonal grasses, as affected by different light supply. *Vegetatio*, 116: 25-32.

Gardner SN & Mangel M (1997) When can a clonal organism escape senescence? *American Naturalist*, 150: 462-490.

Gross N, Suding KN & Lavorel S (2007) Leaf dry matter content and lateral spread predict response to land use change for six subalpine grassland species. *Journal of Vegetation Science*, 18: 289-300.

Hay MJM & Newton PCD (1996) Effect of severity of defoliation on the viability of reproductive and vegetative axillary buds of *Trifolium repens* L. *Annals of Botany*, 78: 117-123.

Hicks S & Turkington R (2000) Compensatory growth of three herbaceous perennial species: the effect of clipping and nutrient availability. *Canadian Journal of Botany*, 78: 759-767.

Huber H, Lukacs S & Watson MA (1999) Spatial structure of stoloniferous herbs: an interplay between structural blue-print, ontogeny and phenotypic plasticity. *Plant Ecology*, 141: 107-115.

Huber H & During HJ (2001) No long-term costs of meristem allocation to flowering in stoloniferous *Trifolium* species. *Evolutionary Ecology*, 14: 731-748.

Hutchings MJ & Bradbury IK (1986) Ecological perspectives on clonal perennial herbs. *BioScience* 36: 178-182.

Hutchings MJ & Wijesinghe D (1997) Patchy habitats, division of labour and growth dividends in clonal plants. *Trends in Ecology and Evolution*, 12: 390-394.

Hutchings MJ (1999) Clonal plants as cooperative systems: benefits in heterogeneous environments. *Plant Species Biology*, 14: 1-10.

Jiaseński M & Bazzaz FA (1999) The fallacy of ratios and the testability of models in biology. *Oikos*, 84: 321-326.

Kleijn D & van Groenendael J (1999) The exploitation of heterogeneity by a clonal plant in habitats with contrasting productivity levels. *Journal of Ecology*, 87: 873-884.

Klimeš L, Klimešovà J, Hendricks R & van Groenendael J (1997) Clonal plant architecture: a comparative analysis of form and function. In: de Kroon H & van Groenendael J (eds) *The ecology and evolution of clonal plants*. Backhuys Publishers, Leiden, pp 1-29.

Klimeš L & Klimešovà J (1999) CLO-PLA2 – A database of clonal plants in central Europe. *Plant Ecology*, 141: 9-19.

Legendre P & Legendre L (1998). *Numerical Ecology*. 2nd English ed. Elsevier, Amsterdam.

Lennartsson T, Nilsson P & Tuomi J (1998) Induction of overcompensation in the field gentian, *Gentianella campestris*. *Ecology*, 79: 1061-1072.

Li B, Shibuya T, Yogo Y & Hara T (2004) Effect of ramet clipping and nutrient availability on growth and biomass allocation of yellow nutsedge. *Ecological Research*, 19: 603-612.

Liao M, Yu F, Song M, Zhang S, Zhang J & Dong M (2003) Plasticity in R/S ratio, morphology and fitness-related traits in response to reciprocal patchiness of light and nutrients in the stoloniferous herb, *Glechoma longituba* L. *Acta Oecologica*, 24: 231-239.

Loucougaray G, Bonis A & Bouzillé JB (2004) Effect of grazing by horses and/or cattle on the diversity of coastal grasslands in western France. *Biological Conservation*, 116: 59-71.

MacDonald SE & Lieffers VJ (1993) Rhizome plasticity and clonal foraging of *Calamagrostis canadensis* in response to habitat heterogeneity. *Journal of Ecology*, 81: 769-776.

Macek P & Lepš J (2003) The effect of environmental heterogeneity on clonal behaviour of *Prunella vulgaris* L. *Plant Ecology*, 168: 31-43.

Maschinski J & Whitham TG (1989) The continuum of plant responses to herbivory: the influence of plant association, nutrient availability, and timing. *American Naturalist*, 134: 1-19.

McNaughton SJ & Chapin III FS (1985) Effects of phosphorus nutrition and defoliation on C_4 graminoids from the Serengeti plains. *Ecology*, 66: 1617-1629.

Moen J, Ingvarsson PK & Walton DWH (1999) Estimates of structural complexity in clonal plant morphology: comparison of grazed and ungrazed *Acaena magellanica* rhizomes. *Canadian Journal of Botany*, 77: 869-876.

Oborny B & Kun A (2002) Fragmentation of clones: how does it influence dispersal and competitive ability? *Evolutionary Ecology*, 15: 319-346.

Owen D (1980) How plants may benefit from the animals that eat them. *Oikos*, 35: 230-235.

Paige KN & Whitham TG (1987) Overcompensation in response to mammalian herbivory: the advantage of being eaten. *American Naturalist*, 129: 407-416.

Piqueras J (1999) Herbivory and ramet performance in the clonal herb *Trientalis europara* L. *Journal of Ecology*, 87: 450-460.

Price EAC & Marshall C (1999) Clonal plants and environmental heterogeneity. An introduction to the proceedings. *Plant Ecology*, 141: 3-7.

Richards JH, Mueller RJ & Mott MT (1988) Tillering in tussock grasses in relation to defoliation and apical bud removal. *Annals of Botany*, 62: 173-179.

Rossignol N, Bonis A & Bouzillé JB (submitted) Short- and long-term effects of grazing intensity on vegetation properties in a wet grassland.

Stowe KA, Marquis RJ, Hochwender CG & Simms EL (2000) The evolutionary ecology of tolerance to consumer damage. *Annual Review of Ecology and Systematics*, 31: 565-595.

Stuefer JF & Huber H (1998) Differential effects of light quantity and spectral light quality on growth, morphology and development of two stoloniferous *Potentilla* species. *Oecologia*, 117: 1-8.

Strauss SY & Agrawal AA (1999) The ecology and evolution of plant tolerance to herbivory. *Trends in Ecology and Evolution*, 14:1 79-185.

Suzuki JI & Stuefer JF (1999) On the ecological and evolutionary significance of storage in clonal plants. *Plant Species Biology*, 14: 11-17.

Thioulouse J, Chessel D, Dodélec S & Olivier JM (1997) ADE-4: a multivariate analysis and graphical display software. *Statistical Computing*, 7: 75-83.

Wang Z, Li L, Han X & Dong M (2004) Do rhizome severing and shoot defoliation affect clonal growth of *Leymus chinensis* at ramet population level ? *Acta Oecologica*, 26: 255-260.

Table S1. Effects of defoliation treatments on traits related to plant clonal performance. Treatment as single factor was tested by ANOVAs for number of ramets and RGR. Treatment as main factor and final biomass as covariate were tested by ANCOVAs for ramet biomass (data log-transformed). ns $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Letters indicate significant differences between treatments (post-hoc Tukey's tests). C: control, M: moderate, S: severe treatments. Abbreviations of species follow Table 2.

	mean ± sd			factors	
	treatment C	treatment M	treatment S	treatment	final biomass
A – Number of ramets					
Asto	88.0 ± 50.6	59.8 ± 28.2	87.9 ± 33.7	$F^2_{30} = 1.94$ ns	
Cdiv	5.1 ± 1.7	4.6 ± 1.6	4.1 ± 2.2	$F^2_{27} = 0.73$ ns	
Epal	23.3 ± 18.2^{a}	10.6 ± 5.6^{b}	6.6 ± 5.2^{b}	$F^2_{28} = 6.88$ **	
Erep	21.3 ± 6.1	17.1 ± 12.6	17.4 ± 8.2	$F^2_{30} = 0.99$ ns	
Gflu	31.9 ± 10.8^{a}	16.3 ± 4.9^{b}	24.8 ± 8.5^{ab}	$F^2_{29} = 8.78$ **	
Jart	22.6 ± 11.1	21.2 ± 10.0	21.3 ± 11.6	$F^2_{28} = 0.00$ ns	
Jger	5.8 ± 3.8	4.8 ± 2.0	3.6 ± 2.4	$F^2_{25} = 1.40$ ns	
Rrep	10.6 ± 6.2	9.4 ± 5.4	13.6 ± 4.1	$F^2_{27} = 1.65$ ns	
Tfra	38.3 ± 11.0^{a}	28.7 ± 7.1^{ab}	23.5 ± 10.8^{b}	$F^2_{29} = 6.16$ **	
Trep	19.4 ± 8.4	28.2 ± 8.8	23.8 ± 9.0	$F^2_{28} = 2.82$ ns	
B – Relative Growth Rate (RGR)					
Asto	0.057 ± 0.004^{a}	0.049 ± 0.005^{b}	0.049 ± 0.006^{b}	$F^2_{30} = 10.26$ ***	
Cdiv	0.026 ± 0.005^{a}	0.020 ± 0.007^{ab}	0.016 ± 0.007^{b}	$F^2_{27} = 7.11$ **	
Epal	0.024 ± 0.004^{a}	0.019 ± 0.004^{b}	0.016 ± 0.003^{b}	$F^2_{28} = 15.36$ ***	
Erep	0.049 ± 0.004^{a}	0.038 ± 0.008^{b}	0.038 ± 0.007^{b}	$F^2_{30} = 10.59$ ***	
Gflu	0.041 ± 0.005^{a}	0.029 ± 0.004^{b}	0.028 ± 0.006^{b}	$F^2_{29} = 20.97$ ***	
Jart	0.042 ± 0.007	0.037 ± 0.005	0.037 ± 0.005	$F^2_{28} = 3.25$ ns	
Jger	0.030 ± 0.008	0.026 ± 0.007	0.024 ± 0.009	$F^2_{25} = 1.43$ ns	
Rrep	0.037 ± 0.005^{a}	0.033 ± 0.005^{ab}	0.030 ± 0.004^{b}	$F^2_{27} = 6.19$ **	
Tfra	0.060 ± 0.004^{a}	0.056 ± 0.003^{a}	0.049 ± 0.005^{b}	$F^2_{29} = 19.64$ ***	
Trep	0.059 ± 0.003^{a}	0.056 ± 0.005^{ab}	0.052 ± 0.005^{b}	$F^2_{28} = 6.32$ **	
C – Above-ground biomass of ramets (g)					
Asto	2.24 ± 0.93	0.93 ± 0.47	0.69 ± 0.28	$F^2_{29} = 0.41$ ns	$F^1_{29} = 53.32$ ***
Cdiv	0.77 ± 0.36	0.43 ± 0.13	0.30 ± 0.10	$F^2_{26} = 0.20$ ns	$F^1_{26} = 115.09$ ***
Epal	1.23 ± 0.52	0.64 ± 0.28	0.39 ± 0.22	$F^2_{27} = 0.08$ ns	$F^1_{27} = 230.90$ ***
Erep	2.83 ± 1.07	1.01 ± 0.59	0.59 ± 0.24	$F^2_{29} = 0.63$ ns	$F^1_{29} = 367.28$ ***
Gflu	2.46 ± 0.10	0.81 ± 0.24	0.74 ± 0.18	$F^2_{28} = 0.20$ ns	$F^1_{28} = 25.56$ ***
Jart	1.66 ± 0.82	0.10 ± 0.47	0.73 ± 0.22	$F^2_{27} = 1.45$ ns	$F^1_{27} = 397.68$ ***
Jger	0.33 ± 0.18	0.22 ± 0.15	0.17 ± 0.06	$F^2_{24} = 0.72$ ns	$F^1_{24} = 144.26$ ***
Rrep	3.43 ± 1.46	1.60 ± 0.88	1.23 ± 0.46	$F^2_{26} = 1.17$ ns	$F^1_{26} = 71.37$ ***
Tfra	0.21 ± 0.08	0.13 ± 0.05	0.11 ± 0.08	$F^2_{28} = 1.97$ ns	$F^1_{28} = 3.42$ *ns*
Trep	0.09 ± 0.05^{b}	0.14 ± 0.04^{a}	0.10 ± 0.05^{a}	$F^2_{27} = 8.29$ **	$F^1_{27} = 12.42$ **

Table S2. Effects of defoliation treatments on clonal-architecture-related traits. Treatment as single factor was tested by non-parametric Kruskal-Wallis' tests for number of connections and ANOVAs for total length of connections and mean internode length (data log-transformed). See table 3 for legend, no: non observed.

	mean ± sd			treatment effect
	treatment C	treatment M	treatment S	
A – Number of connections				
Asto	16.5 ± 8.1	16.8 ± 8.0	25.5 ± 10.9	H^2_{30} = 4.52 ns
Cdiv	2.1 ± 1.7^{a}	1.1 ± 0.5^{ab}	0.9 ± 0.3^{b}	H^2_{27} = 6.12 *
Epal	4.1 ± 3.3^{a}	1.3 ± 1.0^{b}	0.8 ± 0.4^{b}	H^2_{28} = 13.09 **
Erep	7.0 ± 3.1^{a}	3.3 ± 1.7^{b}	3.6 ± 2.2^{b}	H^2_{30} = 11.79 **
Gflu	2.3 ± 2.3	1.6 ± 1.2	0.7 ± 1.0	H^2_{29} = 4.43 ns
Jart	4.2 ± 2.1	5.0 ± 1.8	4.6 ± 1.7	H^2_{28} = 0.29 ns
Jger	1.7 ± 1.0	1.1 ± 0.3	1.0 ± 0.5	H^2_{25} = 3.24 ns
Rrep	2.8 ± 1.1	2.6 ± 1.7	3.6 ± 1.0	H^2_{27} = 3.95 ns
Tfra	16.5 ± 4.7^{a}	13.9 ± 4.5^{a}	8.6 ± 3.3^{b}	H^2_{29} = 12.31 **
Trep	27.7 ± 8.5	25.1 ± 7.0	19.3 ± 7.6	H^2_{28} = 5.05 ns
B – Mean length of connections (cm)				
Asto	32.5 ± 7.0^{a}	14.7 ± 3.9^{b}	7.9 ± 1.2^{c}	F^2_{30} = 120.04 ***
Cdiv	4.3 ± 1.7^{a}	3.3 ± 1.3^{ab}	2.3 ± 1.1^{b}	F^2_{24} = 5.31 *
Epal	8.4 ± 3.0	10.1 ± 3.4	8.8 ± 8.9	F^2_{25} = 1.25 ns
Erep	9.8 ± 2.9	14.8 ± 4.8	13.9 ± 2.3	F^2_{29} = 1.86 ns
Gflu	19.4 ± 5.9^{a}	10.6 ± 2.6^{b}	9.0 ± 0.2^{b}	F^2_{19} = 17.02 ***
Jart	1.4 ± 0.3	1.3 ± 0.4	1.2 ± 0.3	F^2_{28} = 1.11 ns
Jger	2.2 ± 1.5	2.6 ± 1.7	2.2 ± 0.5	F^2_{25} = 0.26 ns
Rrep	42.7 ± 15.4	41.6 ± 20.6	44.0 ± 11.3	F^2_{27} = 0.22 ns
Tfra	9.9 ± 2.7	9.7 ± 2.2	9.4 ± 2.2	F^2_{29} = 0.11 ns
Trep	7.6 ± 1.0	6.9 ± 0.9	6.8 ± 1.7	F^2_{28} = 1.50 ns
C – Mean inter-ramet distance (cm)				
Asto	4.6 ± 1.5^{a}	2.5 ± 0.5^{b}	2.1 ± 0.3^{b}	F^2_{30} = 29.89 ***
Cdiv	1.1 ± 0.6^{a}	0.5 ± 0.2^{b}	0.4 ± 0.2^{b}	F^2_{24} = 9.98 ***
Epal	1.0 ± 0.4	1.0 ± 0.4	0.8 ± 0.5	F^2_{24} = 0.89 ns
Erep	no	no	no	
Gflu	no	no	no	
Jart	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.2	F^2_{28} = 0.10 ns
Jger	0.3 ± 0.1	0.2 ± 0.1	0.4 ± 0.3	F^2_{22} = 1.27 ns
Rrep	9.3 ± 3.7	9.1 ± 2.0	8.7 ± 1.5	F^2_{27} = 0.07 ns
Tfra	1.8 ± 0.6	1.9 ± 0.8	1.5 ± 0.3	F^2_{29} = 1.42 ns
Trep	1.8 ± 0.2^{a}	1.3 ± 1.2^{b}	1.1 ± 0.3^{b}	F^2_{28} = 21.69 ***

Article 5 – Spatial patterns of clonal fragments and architectural responses to defoliation depend on the structural blue-print: an experimental evidence.

Marie-Lise Benot, Anne Bonis & Cendrine Mony

UMR CNRS 6553, ECOBIO, Université de Rennes 1, Campus de Beaulieu, 35042 Rennes Cedex, France

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Abstract

Clonal architecture is involved in the performance of clonal fragments, as it determines the spatial distribution of ramets. It is expected to rely on the species-specific expression of several architectural traits (structural blue-print). However, in contrasted environments, realized clonal architectures may differ, due to phenotypic plasticity. In grazed meadows, clonal fragments are likely submitted to defoliation, which may generate plastic changes in clonal architecture. While losses of biomass may limit lateral expansion, the release of apical dominance and decrease in inter-ramet competition are may enhance branching and ramet production. We tested the hypotheses that (1) exploration and occupation of space depend on the expression of several species-specific architectural traits, and that (2) plastic response of these traits to defoliation leads to a more compact architecture. We compared clonal architectures between two rhizomatous ecologically close Cyperaceae (*Carex divisa* and *Eleocharis palustris*) in undisturbed conditions and when defoliated. Traits related to clonal performance and architecture were monitored through an original non-destructive mapping method. In undisturbed conditions, both species showed similar production of biomass but contrasted architectures and spatial patterns. The rhizome network of *C. divisa*, which consisted in only two primary rhizomes but several branches, was involved in resource storage rather than in spatial colonization. By contrast, *E. palustris* produced on average six primary rhizomes that grew in the whole horizontal plan, maximizing occupation and exploration of space. Both species showed also differential responses to defoliation. In *C. divisa*, the costs associated to defoliation caused a decrease in branching, limiting the area occupied and the number of ramets produced by clonal fragments. By contrast, the weakly branched rhizome networks of *E. palustris* were not affected by defoliation. We concluded on the potential advantages provided by both strategies of rhizome growth (storage *vs*. spatial expansion) in grazed meadows.

Key words

Carex divisa; *Eleocharis palustris*; exploration of space; mapping; occupation of space; rhizome network.

Introduction

Clonal growth relies on the iterative production of potentially autonomous descendents, the ramets, linked altogether by plagiotropic stem-derived connections, either above-ground or below-ground (*stolons* and *rhizomes*, respectively). Clonal growth thus confers on plants the ability to spread horizontally. While this property enables clonal fragments to proliferate and colonize space (Hutchings & Mogie 1990), it may also generate competition between ramets of a same clonal fragment, as ramets develop vertical structures close to each other (Herben & Hara 1997). Consequently, the spatial distribution of ramets within a clonal fragment is a key feature of clonal plants, as it determines the magnitude of inter-ramet competition and access to resources (Huber *et al*. 1999), as well as the interactions between clonal fragments (Herben & Hara 1997). In rhizomatous species, above-ground spatial patterns may depend on the characteristics of below-ground structures. Yet, a handful of empirical studies investigated the architecture of rhizome networks, notably because it is more difficult to measure than the spatial structure of stolons (Meyer & Schmid 1999; Sammul *et al*. 2003, 2004; Wildová 2004; Wildová *et al*. 2007b).

Clonal architectures can be organized along a gradient ranging from *guerrilla* to *phalanx* growth forms (Lovett-Doust 1981). Guerrilla growth forms are characterized by long connections and spacers (*i.e.* fractions of connections between consecutive ramets; Bell 1984), enhancing lateral expansion and the area covered by the clonal fragment (*i.e. exploration* of space). In phalanx growth forms, highly branched rhizome networks and short spacers maximize the aggregation of ramets in relatively small areas (*i.e. occupation* of space). Through a geometry model, Smith & Palmer (1976) predicted that an optimal architecture would maximize both horizontal spreading and ramet density. Therefore, this type of architecture would be intermediate between guerrilla and phalanx growth forms. However, clonal architectural patterns are diverse, depending on intrinsic and extrinsic factors, and do not always correspond to this optimum (Lovett-Doust 1981; Bell 1984).

On the one hand, in unlimited and undisturbed environments, the architecture of a clonal fragment at a given development stage is expected to depend on species-specific structural constraints such as the number and activity of buds available (*i.e.* structural blue-print; Bell 1984; Huber *et al*. 1999). In particular, it is likely to rely on the species-specific expression of several architectural traits (*e.g.* number and length of connections, length of spacers, branching angles). However, the relative importance of these traits in the spatial dynamics of clonal fragments remains unclear (see for instance Sutherland & Stillman 1988; Cain 1994; Wildová *et al*. 2007a).

On the other hand, in contrasted environments, clonal fragments of a same species may show morphological and architectural differences. The expression of architectural traits will not only rely on species-specific structural blue-print, but will also be modulated by phenotypic plasticity in response to the environment (Huber *et al*. 1999). In particular, clonal architecture-related traits have been predicted to respond to habitat quality. The decrease in the length of connections and spacers, and/or the increase in rhizome branching are expected to promote the aggregation of ramets in favourable habitats. This type of foraging property has been empirically demonstrated in response to light, nutrient and water availability, or competition (see for instance Slade & Hutchings 1987a,b; Price & Hutchings 1996; for studies on *Glechoma hederacea*, and references therein), a favourable habitat corresponding to a resource-rich or competition-free one.

In meadows, grazing is a complex factor that affects vegetation through several ways, one of the most important being defoliation (Kohler *et al*. 2004). Although plastic responses of clonal architecture to defoliation undoubtedly influence species abundance and clonal diversity in meadow communities, they have rarely been studied (see however Price & Hutchings 1992b; Moen *et al*. 1999; Hay & Newton 1996; Wang *et al*. 2004). In the particular case of defoliation, defining the quality of the habitat is tricky, as defoliation generates at the same time losses of above-ground tissues (disturbance, *sensu* Grime 1977) and decrease in above-ground competition through canopy opening. Injuries caused by defoliation and compensatory growth following defoliation are costly to the plant (van der Meijden *et al*. 1988) and may divert resources from current clonal growth. Thus, defoliation can be expected to reduce clonal expansion and to disfavour guerrilla growth forms. Indeed, experimental defoliation has already been demonstrated to decrease connection length (Price & Hutchings 1992b; Wang *et al*. 2004). Defoliation may also release apical dominance, which is likely to result in the activation of axillary buds and to promote regeneration following damage (Tuomi *et al.* 1994). In clonal plants, the activation of vegetative buds could enhance branching and the production of ramets (Klimešová & Klimeš 2003, 2007). Moreover, the decrease in inter-ramet competition for light should allow the aggregation of ramets, leading to more compact (*i.e.* phalanx-like) architectures.

The objective of this study was to disentangle the relevance of a set of architectural traits in the effective clonal architecture of clonal fragments grown in undisturbed and unlimited conditions, and to describe their plastic responses to defoliation. In that purpose, we carried out an experiment on two rhizomatous Cyperaceae species: *Carex divisa* Huds. and *Eleocharis palustris* Rœm. & Schult. These species are present in similar abundances in

wetland pastures of the Marais Poitevin (Western France), where they occur all along a grazing gradient going from no to heavy grazing (Loucougaray *et al*. 2004). We particularly tested the following hypotheses. (1) In undisturbed and unlimited environmental conditions, exploration and occupation of space depend on the species-specific expression of several architectural traits, reflecting the species-specific structural blue-print. (2) Defoliated clonal fragments enhance occupation instead of exploration of space, through compact (*e.g.* phalanx-like) over dispersed (*e.g.* guerrilla-like) clonal architectures.

Materials and Methods

Experimental design

We compared the architectural characteristics between both species and the response of architectural traits to defoliation by testing two defoliation treatments: no cutting (control treatment) and recurrent cutting (defoliation treatment).

In September 2005, thirty clonal fragments of *C. divisa* and *E. palustris* were randomly sampled at a minimal distance of five meters from each other, in grazed meadows in the Marais Poitevin, Western France (46°28′N, 1°30′W). They were then transplanted in an experimental garden at the University of Rennes (France), where they were grown for six months in undisturbed conditions and in rich garden soil that was watered daily. On 8 March 2006, we selected 14 ramets per species from 14 different clonal fragments, cleaned them and weighed them for fresh mass. We deduced their initial dry mass from the linear regression between fresh mass and dry mass using 12 additional ramets per species. Ramets were planted in the centre of 80-cm × 80-cm × 15-cm culture plots containing fine and rich garden soil, located in an outdoor experimental garden. Plots were watered daily with tap water, and the weeds were regularly removed.

We established seven replicates per species × treatment situation and randomly assigned each combination to culture plots. The defoliation treatment consisted of cutting the above-ground parts of each ramet at a height of 5-cm every 15 days. The clipped tissues were dried at 60°C to constant mass and weighed. We applied the first defoliation on 12 May 2006, after 65 days of growth. At this point, clonal fragments were composed of one daughter ramet in addition to the planted one. The experiment lasted for 18 weeks starting from the first defoliation. In the defoliation treatment, the clonal fragments were cut eight times and harvested three weeks after the last defoliation event.

Assessment of clonal architecture and growth

At the end of experiment, we carefully removed the soil covering the rhizome system, without altering the position of the plants. We marked the position of all ramets using coloured pins placed near the ramet base (Fig. 1a). We took a photograph of each clonal fragment in order to describe the pattern of clonal growth in detail. For this purpose, we fixed a camera 1.30 m above each plot using a wooden frame. We corrected the optical distortions of the photographs considering the standard parameters of the camera. We imported the photographs into GIS software (ArgGis 9 software) and calculated the spatial coordinates (x, y) of all ramets using the plot as the referential (Fig. 1b). We digitalized the *spacers* (*i.e.* the fraction of rhizome separating two consecutive ramets; Bell 1984) as straight lines (Fig. 1c). Consequently, the rhizomes were digitalized as polylines linking ramets. We considered the rhizomes developing from the parent ramet as primary rhizomes and the rhizomes branching from a primary or a higher-order rhizome as branches (Fig. 1c).

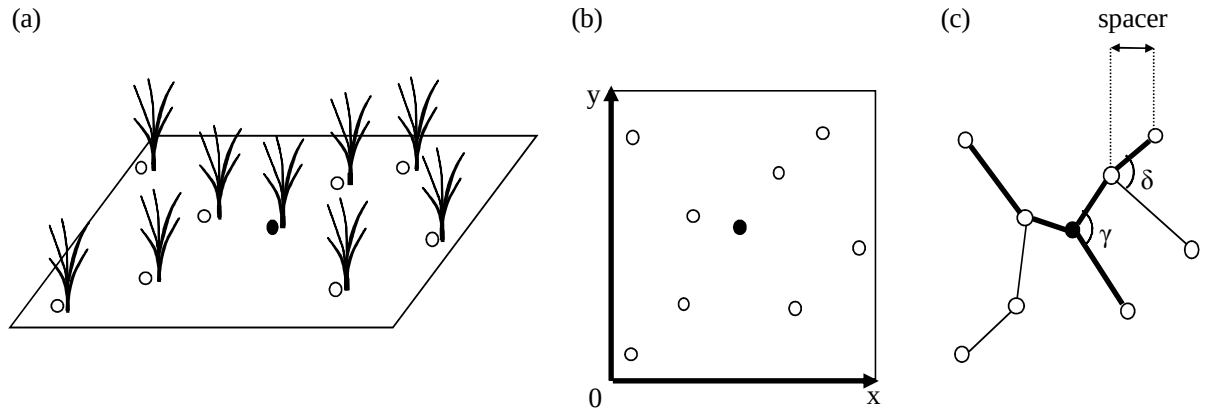

Fig. 1. Measurement of clonal architecture. (a) Schematic representation of a clonal fragment in an experimental plot. Circles represent the pins used to mark the position of each ramet. Black circle: parent ramet, empty circles: daughter ramets. (b) Position of ramets in the horizontal plan of the plot referential. (c) Clonal architecture. Bold lines: primary rhizomes, thin lines: branches, γ: angles between primary rhizomes, δ: branching angle.

From these maps of clonal fragments, we extracted the number of ramets, area covered by the clonal fragment and traits related to the architectural pattern of the clonal fragment. At the end of the experiment, we harvested entire clonal fragments (all above-ground and below-ground organs), dried them at 60°C to constant mass and weighed them. Investigated traits were grouped into two categories, which were:

1) Traits indicative of clonal performance:

- Total biomass of the clonal fragment, calculated as the sum of final biomass of the whole clonal fragment and biomass of tissues clipped during the experiment,
- Mean biomass of ramets, calculated as the sum of final biomass of ramets and biomass of clipped tissues, and divided by the number of ramets,
- Number of ramets per clonal fragment, which enabled to evaluate the *occupation* of space, and
- Area occupied by the clonal fragment, which was used to estimate *exploration* of space. It was calculated by the local nearest-neighbour convex-hull construction (Getz and Wilmers 2004)

2) Traits related to clonal architecture:
   - Total length of rhizomes, calculated as the sum of the lengths of all primary rhizomes and branches,
   - Density of rhizomes, calculated as the biomass of rhizomes divided by total length of rhizomes,
   - Number of primary rhizomes and branches,
   - Mean length of primary rhizomes and branches,
   - Mean length of spacers on primary rhizomes and branches, and
   - Mean angle between rhizomes.

All of the spatial treatments were carried out using the ArgGis 9 software.

Statistical analyses

For both species, General Linear Model (GLM) ANOVAs showed no differences in the initial dry mass of ramets between both treatments, confirming the assumption of ramet randomization between the treatments at the beginning of the experiment ($F_{1,12} = 0.93$, $P = 0.35$ for *C. divisa*, and $F_{1,12} = 1.46$, $P = 0.25$ for *E. palustris*).

Inter-specific differences in (i) biomass of tissues clipped during the experiment and (ii) traits of control clonal fragments (*i.e.* clonal fragments that had not been submitted to defoliation) were tested by linear models with species as the main factor, total biomass (sum of the final biomass of the clonal fragment and biomass of clipped tissues) as the covariate, and their interaction. For both species, trait responses to defoliation were analyzed by linear models with defoliation treatment as the main factor, total biomass as the covariate, and their interaction. GLM ANOVAs were applied for weights, lengths and angles. Data were log-transformed for normalization when necessary. Generalized Linear Model with Poisson error distribution (GLIM) ANOVAs were applied for numbers. We used total biomass as a

covariate in these analyses in order to take the growth capacity of the clonal fragments into account. The statistical analyses were carried out with the R software (R Development Core Team, 2007, http://www.R-project.org).

Results

Inter-specific comparison of performance and clonal architecture

Examples of the architecture of clonal fragments for both species are given in Figure 2.

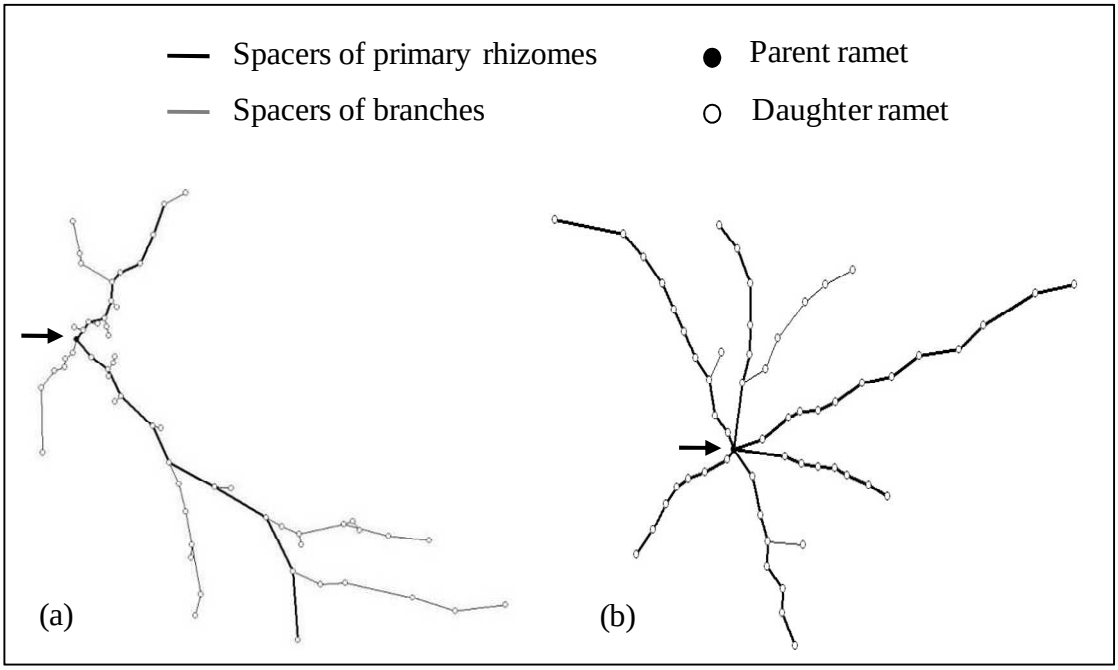

Fig. 2. Examples of GIS representations of clonal architecture. (a) *Carex divisa*. (b) *Eleocharis palustris*. Arrows indicate the position of the parent ramet.

Total biomass and mean biomass of ramets were similar for both species, but *E. palustris* fragments displayed a higher number of ramets and occupied a significantly wider area than *C. divisa* fragments (Tables 1; 2). The mean biomass of ramets, density of rhizomes, number of primary rhizomes and angles between the rhizomes were not related to total biomass (Table 2), indicating that these traits were not linked to the growth potential of the plants. Although the difference was only marginally significant, total length of rhizomes reached on average less than 0.9 m for *C. divisa* compared to up to 1.7 m for *E. palustris* (Fig. 3a; Table 2). By contrast, density of rhizomes in *C. divisa* was about 2.5 times greater than in *E. palustris* (Fig. 3b; Table 2). The architectural differences between both species mostly depended on the number and characteristics of the primary rhizomes (Table 2).

Table 1. Means ± SE (n = 7) of traits related to clonal performance, for both species and defoliation treatments.

	C.divisa		*E.palustris*	
	Control	Defoliation	Control	Defoliation
Total biomass (g)	24.83 ± 6.01	11.59 ± 1.53	28.61 ± 6.19	28.92 ± 2.38
Mean biomass of ramets (g)	0.20 ± 0.03	0.18 ± 0.04	0.18 ± 0.03	0.18 ± 0.02
Number of ramets	52.3 ± 7.4	34.9 ± 4.0	99.6 ± 31.6	88.6 ± 11.6
Area (m^2)	0.031 ± 0.008	0.009 ± 0.002	0.088 ± 0.045	0.055 ± 0.012

Table 2. Results of ANOVAs (*P*-values) testing for the inter-specific differences of trait values. Significant results are in bold. Italic letters indicate the model applied: *g*, GLM; *gt*, GLM on log-transformed data; *gp*, GLIM with Poisson error distribution.

	Species	Total biomass	Species × total biomass	
Performance				
Total biomass	0.503	/	/	*gt*
Mean biomass of ramets	0.522	0.699	**0.005**	*g*
Number of ramets	< **0.001**	< **0.001**	< **0.001**	*gp*
Area	< **0.001**	< **0.001**	< **0.001**	*g*
Architecture				
Total length of rhizomes	0.068	< **0.001**	0.061	*gt*
Density of rhizomes	< **0.001**	0.579	0.051	*g*
Number of rhizomes				
Primary rhizomes	< **0.001**	0.969	0.463	*gp*
Branches	0.153	< **0.001**	< **0.001**	*gp*
Mean angle between rhizomes				
Primary rhizomes (γ)	**0.024**	0.536	0.198	*g*
Branches (δ)	0.469	0.167	0.576	*g*
Mean length of rhizomes				
Primary rhizomes	0.185	**0.010**	0.126	*gt*
Branches	0.115	< **0.001**	0.064	*g*
Mean length of spacers				
Primary rhizomes	**0.009**	**0.003**	0.189	*gt*
Branches	0.755	< **0.001**	0.664	*g*

C. divisa fragments tended to grow directionally (Fig. 2a). They produced on average two primary rhizomes spaced by about 108° (Fig. 3c,d). These rhizomes produced an average of 14 branches per clonal fragment (Fig. 3g). On the contrary, *E. palustris* colonized space in all of the horizontal directions (Fig. 2b) as clonal fragments produced about six primary rhizomes spaced by 65° angles (Fig. 3c,d). Despite the greater number of primary rhizomes,

they produced as many branches as *C. divisa* (Fig. 3g), with a similar branching angle of about 65° (Fig. 3h). Neither primary rhizomes nor branches significantly differed in mean length between both species (Fig. 3e,i). Ramets on primary rhizomes were significantly more spaced in *C. divisa* than in *E. palustris*, whereas spacers of branches were of similar length between both species (Fig. 3f,j).

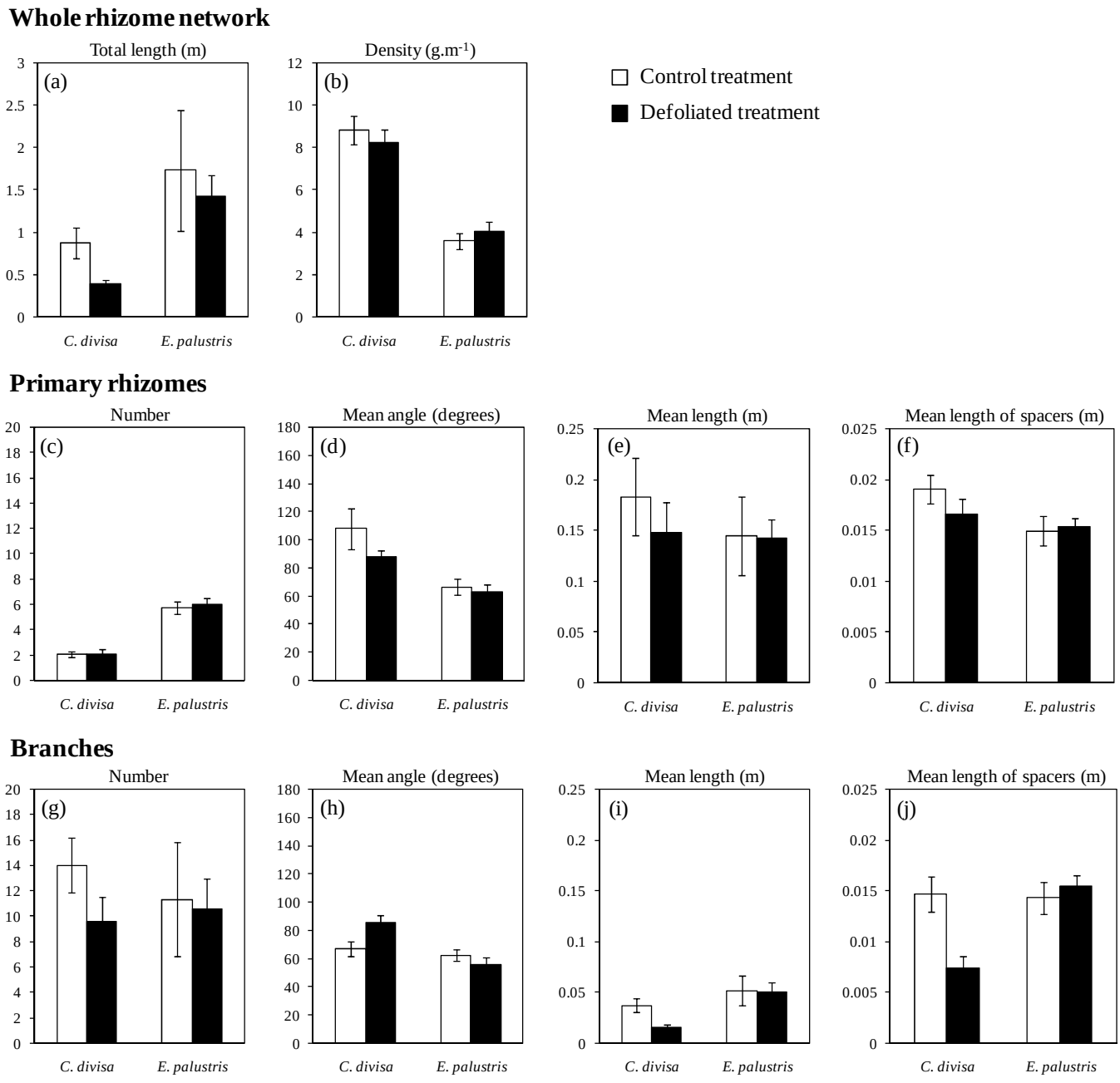

Fig. 3. Mean (± SE) architectural traits for both species and both treatments of defoliation. (a – b) Whole rhizome network (primary rhizomes and branches), (c – f) primary rhizomes, (g – j) branches. Statistical analyses are given in Tables 2 and 3.

Impact of defoliation on performance and clonal architecture

The amount of biomass removed by clipping depended on total biomass of clonal fragments, but was similar for both species (1.96 ± 0.24 g for *C. divisa* and 2.00 ± 0.30 g for *E. palustris*; species effect: $F_{1,10} = 0.07$; $P = 0.81$; total biomass effect: $F_{1,10} = 35.07$; $P = 1.4 \times 10^{-4}$; species × total biomass effect: $F_{1,10} = 10^{-4}$; $P = 0.99$).

In *C. divisa*, defoliation seemed to decrease total biomass (Table 1) but this difference was not significant (Table 3). Indeed, neither total biomass nor mean biomass of ramets were significantly impacted by defoliation in both species (Tables 1; 3). In *C. divisa* defoliation decreased the total number of ramets by 33 % and the area by up to 70 %. By contrast, defoliation only decreased the total number of ramets by 10 % in *E. palustris* (Tables 1; 3).

In *C. divisa*, defoliation impacted traits related to branches (Fig. 3; Table 3). It caused a reduction in branching intensity (the number of branches was reduced by 31 %; Fig. 3g), mean length of branches (58 % decrease; Fig. 3i) and mean length of spacers on branches (50 % decrease; Fig. 3j). In addition, the mean branching angle increased from 67° for the control clonal fragments to 85° for the defoliated ones, leading to branches growing almost perpendicularly to the primary rhizomes (Fig. 3f). By contrast, defoliation affected none architectural trait in *E. palustris* (Fig. 3; Table 3).

Table 3. Results of ANOVAs (*P*-values) testing for the impact of defoliation treatment on traits values for *C. divisa* and *E. palustris*. Significant results are in bold. Italic letters indicate the model applied: *g*, GLM; *gt*, GLM on log-transformed data; *gp*, GLIM with Poisson error distribution.

	C. divisa				*E. palustris*			
	Treatment	Total biomass	Treatment × total biomass		Treatment	Total biomass	Treatment × total biomass	
Performance								
Total biomass	0.135	/	/	*gt*	0.605	/	/	*gt*
Mean biomass of ramets	0.352	< **0.001**	0.373	*g*	0.826	0.064	0.717	*g*
Number of ramets	< **0.001**	< **0.001**	0.223	*gp*	**0.030**	< **0.001**	0.580	*gp*
Area	< **0.001**	< **0.001**	0.986	*g*	0.071	< **0.001**	0.075	*g*
Architecture								
Total length of rhizomes	< **0.001**	< **0.001**	0.973	*g*	0.249	< **0.001**	0.144	*g*
Density of rhizomes	0.488	0.106	0.465	*g*	0.420	0.055	0.159	*g*
Number of rhizomes								
Primary rhizomes	1.000	0.434	0.639	*gp*	0.825	0.529	0.510	*gp*
Branches	**0.015**	**0.010**	0.826	*gp*	0.686	< **0.001**	0.235	*gp*
Mean angle between rhizomes								
Primary rhizomes (γ)	0.360	0.237	0.821	*g*	0.639	0.123	0.160	*g*
Branches (δ)	**0.011**	0.060	0.056	*g*	0.342	0.784	0.330	*g*
Mean length of rhizomes								
Primary rhizomes	0.511	0.345	0.971	*gt*	0.437	< **0.001**	0.834	*gt*
Branches	**0.001**	**0.002**	0.645	*g*	0.928	**0.005**	0.403	*g*
Mean length of spacers								
Primary rhizomes	0.229	0.197	0.777	*gt*	0.641	< **0.001**	0.404	*g*
Branches	< **0.001**	< **0.001**	0.175	*g*	0.408	**0.003**	0.805	*g*

Discussion

Our results confirmed that inter-specific differences in the expression of architectural traits resulted in contrasted exploration and occupation of space (first hypothesis). The species-specific structural blue-print also constrained phenotypic plasticity. Contrary to our second hypothesis, defoliation did not lead to a more compact architecture, enhancing occupation of space. By contrast, both species diverged in their response to defoliation. *C. divisa* showed a plastic response, with a decrease in rhizome branching and ramet production, whereas all of the architectural traits of *E. palustris* were maintained.

Inter-specific comparison of clonal architecture, exploration and occupation of space, in non-defoliated conditions

Although total biomass of clonal fragments and mean biomass of ramets were similar for *C. divisa* and *E. palustris*, the expression of several clonal architecture-related traits varied between these two rhizomatous Cypereaceae, leading to different spatial patterns of rhizome networks. These observations are in accordance with Schmid & Bazzaz (1990), who also described contrasted clonal architectures between *Aster* sp. and *Solidago* sp., two rhizomatous Asteraceae, while clonal fragments were of similar size.

C. divisa fragments produced on average only two primary rhizomes, but rather invested in branching (up to seven branches per primary rhizomes). In root systems, highly branched networks have been suggested to decrease exploitation efficiency, as branches are likely to develop in the depletion zone of their parent root, and thus to compete with it (Fitter 1987; Fitter *et al*. 1991). A similar trend could be expected for rhizome networks, in which a great number of branches could enhance inter-ramet competition. This is all the more true for *C. divisa* as the primary rhizomes are separated by angles of 108°, constraining the clonal fragment to grow directionally and to explore a limited area of the whole horizontal plan. Great spacer lengths recorded on primary rhizomes could be advantageous in this kind of architecture, as they are expected to decrease competition between ramets (Herben & Suzuki 2002). In counterpart, they might reduce ramet density and occupation of space (Herben & Suzuki 2002), while demanding an important investment the production of rhizomes. The architectural pattern of *C. divisa* led to a lower spatial exploration and the production of fewer ramets than *E. palustris*. However, rhizomes of *C. divisa* were denser than those of *E. palustris*. In addition to their involvement in spatial expansion, rhizomes can serve as storage organs (Dong & de Kroon 1994; Suzuki & Stuefer 1999). The greater density but smaller length of rhizomes in *C. divisa* than in *E. palustris* suggested the existence of a trade-

off between both functions. The rhizome network of *C. divisa* appeared to be involved in reserve making rather than in spatial exploration, while *E. palustris* presented the inverse pattern.

Contrasting with *C. divisa*, *E. palustris* clonal fragments produced on average six primary rhizomes, which grew in the whole horizontal plan and produced a few branches. Angles between the primary rhizomes and also between branches averaged 65°. In a simulation study, Wildová *et al*. (2007a) demonstrated that changes in branching angles had an inconsistent effect on the performance of clonal fragments, whether measured as the above-ground biomass or the final number of ramets. On the contrary, Bell (1979) suggested that the angles governed the exploration of space and thus, had an ecological significance. More precisely, the simulation model of Smith & Palmer (1976) demonstrated that a hexagonal architecture (*i.e.* a honeycomb-like architecture, with 120° angles between the rhizomes) would maximize the centrifugal spread and area occupied by the clonal fragment, while generating gaps within it. They proposed that the production of branches into these gaps, leading to a 60° branching pattern, would be optimal as it would lead to a population of ramets dense enough to prevent the intrusion of competitors, while limiting the investment in the rhizome network. A weak variation of a few degrees of these angles, as found in our study, would furthermore decrease the risk of ramet superposition (Bell & Tomlinson 1980; Callaghan *et al*. 1990; Meyer & Schmid 1999). A similar pattern has already been observed in other rhizomatous species such as *Solidago altissima* (Meyer & Schmid 1999) or the seagrass *Zostera noltii* (Brun *et al*. 2007). As predicted by Smith & Palmer's model (1976), these architectural characteristics generated a clumped growth form that led to the colonization of a wider area and the production of a greater number of ramets than in *C. divisa*.

The expression of architectural traits related to the length of rhizomes and spacers as well as to the number of branches, was size-dependent. Such results are in accordance with the relationship between the size of a clonal fragment and the structure of its rhizome network observed in *Solidago altissima* (Meyer & Schmid 1999). Through allometric effects, the size of clonal fragments thus modulated the expression of the species-specific architectural traits (Huber & Stuefer 1997). By contrast, in both species, the number of primary rhizomes and angles between the rhizomes were not correlated with plant biomass, suggesting that they did not depend on the size and growth capacity of the clonal fragment, but that they were strictly determined by the structural blue-print. In a given species, the shapes of primary rhizome networks were similar, irrespective of the size of the clonal fragments. These constraints on the growth pattern of primary rhizomes were the main cause of inter-specific differences in

clonal architecture. In *C. divisa*, clonal architecture relied mainly on the production of several branches rather than primary rhizomes and led to directional growth; whereas *E. palustris* fragments produced several but loosely branched primary rhizomes that grew in all directions of the horizontal plan. By contrast, the mean lengths of the primary rhizomes and branches were similar between both species. Therefore, our results provided evidence that inter-specific differences in clonal traits, reflecting the structural blue-print, constrained the whole morphology of the clonal fragment, and as a result, its ability to explore and occupy space.

Impact of defoliation on clonal architecture, exploration and occupation of space

In both species investigated, defoliated clonal fragments proved able to maintain their production of total biomass, as well as the allocation of biomass to ramets. However, our results showed that architectural responses to defoliation were species-specific: while clonal architecture proved plastic in *C. divisa*, all of the architectural traits were maintained in *E. palustris*. These results did not depend on the severity of defoliation, as the amount of biomass removed was equivalent for both species.

Contrasting with increases in tillering (Richards *et al*. 1988) and rhizome branching (Moen *et al*. 1999), which have already been demonstrated in response to defoliation, defoliated clonal fragments of *C. divisa* produced significantly fewer ramets and rhizomes than the control ones. In these clonal fragments, defoliation also caused the decrease of clonal expansion, as shown for other rhizomatous species (Moen *et al*. 1999; Wang *et al*. 2004; Henry *et al*. 2007). This was linked to the decrease in values of traits related to branches: while the number and mean length of the primary rhizomes were maintained, the defoliated clonal fragments produced fewer and shorter branches with shorter spacers. Indeed, clonal growth is costly and requires the allocation of energy, in particular to the production and maintenance of connections (Fisher & van Kleunen 2002). In this species, the losses of biomass induced by defoliation were likely followed by a compensatory growth (as show by the maintained allocation of biomass to ramets), which probably diverted resources from the rhizome network to the shoots (Stoll *et al*. 1998; Wang *et al*. 2004). However, density of rhizomes was maintained in response to defoliation, suggesting that resources stored in these rhizomes were not the substrate used for compensatory growth. Instead of depleting reserves, compensatory growth might have occurred at the expense of the production of branches. These results could explain both the reduced branching caused by defoliation in *C. divisa* and the decrease in the production of new rhizomes caused by mowing in *Solidago altissima* fragments (Meyer & Schmid 1999).

In *C. divisa*, plastic adjustments in response to defoliation also concerned branching angles, which increased from 67° to 85°. The importance of branching angles in plastic responses to the environment has long been discussed, but remains controversial, with modelling studies leading to opposite conclusions (Sutherland & Stillman 1988; Cain 1994). Few empirical studies have addressed this issue. In our study, the increase in branching angles in defoliated *C. divisa* fragments could be related to the costs of defoliation and consecutive compensatory growth. In root systems, angles of 90° appeared to be the shortest way for branches to emerge from the parent root (Fitter 1987), thus demanding less energy. Branching at about 90° from the parent rhizome could accordingly be less costly for the clonal fragment, although it is less efficient in terms of spatial occupation (Fitter 1987). Consequently, plastic response of *C. divisa* fragments to defoliation was orientated towards an economy of energy, at the expense of the production of branches and spatial exploration.

By contrast, the architectural pattern of *E. palustris* appeared highly fixed, as none of the clonal architecture-related traits changed in response to defoliation. However, plasticity in response to defoliation should not be excluded; it may have occurred at other levels (*e.g.* physiological traits) and contributed to the relative stability of the clonal architecture. This strategy enabled the maintenance of the optimal architectural pattern displayed by this species and a maximal exploration of space, even in disturbed conditions. However, defoliation slightly reduced the occupation of space, as proved by a weak but significant decrease in the number of ramets.

In *C. divisa* and *E. palustris*, clonal architecture and the resulting exploration and occupation of space were thus affected by defoliation in contrasted ways. Defoliation impacted branches but not primary rhizomes, the spatial pattern of which proved highly fixed by the species-specific structural blue-print. Consequently, architectures relying mainly on the production of several primary rhizomes and on only a few branches might only be little affected. This was verified by the architectural fixity expressed by *E. palustris* clonal fragments, which contrasted with the decreased investment in branches observed in *C. divisa* clonal fragments. These results demonstrated that species-specific structural blue-print modulated the response to defoliation.

By influencing spatial patterns both below-ground and above-ground, clonal architecture is likely to impact the dynamics of communities dominated by clonal plants, such as grassland vegetation (Briske & Silvertown 1993; Herben *et al*. 2000; Herben & Hara 1997, 2003). In the meadows where they were collected, *C. divisa* and *E. palustris* occur in similar abundances from no grazing at all up to heavy grazing (Loucougaray *et al*. 2004). Thus, our

results provided evidence that different strategies of investment in the rhizome network can lead to similar ecological success. In *E. palustris*, grazing tolerance may in part rely on the relative maintenance of clonal architecture after defoliation. This maintenance may be advantageous as the architectural pattern of *E. palustris* appeared to be optimal and to maximize both exploration and occupation of above-ground and below-ground space, which could even lead to the dominance of the species (Wildová 2004). Yet, *C. divisa* is also commonly present in the study community, despite its different structural blue-print leading to contrasted spatial pattern and response to defoliation. Indeed, in this species, architectural modifications in response to defoliation occurred in the direction of energetic economy and resulted in a reduction of branching and clonal expansion. This plastic response might enable the maintenance of allocation to resource storage and sexual reproduction. Resources stored in rhizomes are protected from grazing-induced defoliation. As they are not mobilized for compensatory growth, they could be used to support ramet production and sexual reproduction at the very beginning of the growing season (Price *et al.* 2002, Wildová 2004, Asaeda *et al.* 2006). Indeed *C. divisa* flowers early before the grazing season and produces long-lived seeds (pers. obs.). Such strategy of below-ground storage rather than spatial exploration and occupation is certainly advantageous in grazed meadows, where it allows both spatial and phenological avoidance of grazing (*sensu* Briske 1996).

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Literature cited

Asaeda T, Rajapakse L, Manatunge J & Sahara N (2006) The effect of summer harvesting of *Phragmites australis* on growrth characteristics and rhizome resource storage. *Hydrobiologia*, 553: 327-335.

Bell AD (1979) The hexagonal branching pattern of rhizomes of *Alpinia speciosa* L. (Zingiberaceae). *Annals of Botany*, 43: 09-223.

Bell AD (1984) Dynamic morphology: a contribution to plant population ecology. In: Dirzo R & Sarukhán J (eds) *Perspectives in Plant Population Ecology.* Sinauer, Sunderland, Massachusetts, pp 48-65.

Bell AD & Tomlinson PB (1980) Adaptive architecture in rhizomatous plants. *Botanical Journal of the Linnean Society*, 80: 125-160.

Briske DD (1996) Strategies of plant survival in grazed systems: a functional interpretation. In: Hodgson J & Illius AW (eds) *The Ecology and management of grazing systems*. CAB International, Wallingford, pp 33-67.

Briske DD & Silvertown JW (1993) Plant demography and grassland community balance: the contribution of population regulation mechanisms. In: Baker MJ (ed) *Proceedings of the XVII International Grassland Congress*. SIR Publishing, Wellington, pp 291-298.

Brun FG, Cummaudo F, Olivé I, Vergara JJ & Pérez-Lloréns JL (2007) Clonal extent, apical dominance and network in features in the phalanx angiosperm *Zostera noltii* Hornem. *Marine Biology*, 151: 1917-1927.

Cain ML (1994) Consequences of foraging in clonal plant species. *Ecology*, 75: 933-944.

Callaghan TV, Svensson BM, Bowmann H, Lindley DK & Carlsson BA (1990) Models of clonal plant growth based on population dynamics and architecture. *Oikos*, 75: 257-269.

Dong M & de Kroon H (1994) Plasticity in morphology and biomass allocation in *Cynodon dactylon*, a grass species forming stolons and rhizomes. *Oikos*, 70: 99-106.

Fischer M & van Kleunen M (2002) On the evolution of clonal plant life histories. Evolutionay *Ecology*, 15: 565-582.

Fitter AH (1987) An architectural approach to the comparative ecology of plant root systems. *New Phytologist*, 105(Suppl): 61-77.

Fitter AH, Stickland TR, Harvey DM & Wilson GW (1991) Architectural analysis of plant root systems. 1. Architectural correlates of exploitation efficiency. *New Phytologist*, 118: 375-382.

Getz WM & Wilmers CC (2004) A local nearest-neighbor convex-hull construction of home ranges and utilization distributions. *Ecography*, 27: 489-505.

Grime JP (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist*, 111: 1169-1194.

Hay MJM & Newton PCD (1996) Effect of severity of defoliation on the viability of reproductive and vegetative axillary buds of *Trifolium repens* L. *Annals of Botany*, 78: 117-123.

Henry GM, Burton MG & Yelverton FH (2007) Effect of mowing on lateral spread and rhizome growth of troublesome *Paspalum* species. *Weed Science*, 55: 486-490.

Herben T, During HJ & Law R (2000) Spatio-temporal patterns in grassland communities. In: Dieckmann U, Law R & Metz JHJ (eds) *The geometry of ecological interactions: simplifying spatial complexity*. Cambridge University Press, New York, pp. 48–64.

Herben T & Hara T (1997) Competition and spatial dynamics of clonal plants. In: de Kroon H & van Groenendael J (eds) *The ecology and evolution of clonal plants*. Backhuys Publishers, Leiden, pp 331-358.

Herben T & Hara T (2003) Spatial pattern formation in plant communities. In: Sekimura T, Noji S, Ueno N & Maini PK (eds) *Morphogenesis and pattern formation in biological systems–experiments and models.* Springer Verlag, Berlin, pp 223–235.

Herben T & Suzuki JI (2002) A simulation study of the effects of architectural constraints and resource translocation on population structure and competition in clonal plants. *Evolutionary Ecology*, 15: 403-423.

Huber H, Lukacs S & Watson MA (1999) Spatial structure of stoloniferous herbs: an interplay between structural blue-print, ontogeny and phenotypic plasticity. *Plant Ecology*, 141: 107-115.

Huber H & Stuefer JF (1997) Shade-induced changes in the branching pattern of a stoloniferous herb: functional response or allometric effect? *Oecologia*, 110: 478-486.

Hutchings MJ & Mogie M (1990) The spatial structure of clonal plants: control and consequences. In: van Groenendael J & de Kroon H (eds) *Clonal growth in plants: regulation and function*. SPB Academic Publishing, The Hague, pp 57-78.

Klimešová J & Klimeš L (2003) Resprouting of herbs in disturbed habitats: is it adequately described by Bellingham - Sparrow's model? *Oikos*, 103: 225-229.

Klimešovà J & Klimeš L (2007) Bud banks and their role in vegetative regeneration – A literature review and proposal for simple classification assessement. *Perspectives in Plant Ecology, Evolution and Systematics*, 8: 115-129.

Kohler F, Gillet F, Gobat JM & Buttler A (2004) Seasonal vegetation changes in mountain pastures due to simulated effects of cattle grazing. *Journal of Vegetation Science*, 15: 143-150.

Loucougaray G, Bonis A & Bouzillé JB (2004) Effect of grazing by horses and/or cattle on the diversity of coastal grasslands in western France. *Biological Conservation*, 116: 59-71.

Lovett-Doust L (1981) Population dynamics and local specialization in a clonal perennial (*Ranunculus repens*). I. The dynamics of ramets in contrasting habitats. *Journal of Ecology*, 69: 743-755.

Meyer AH & Schmid B (1999) Experimental demography of rhizome populations of establishing clones of *Solidago altissima*. *Journal of Ecology*, 87: 42-54.

Moen J, Ingvarsson PK & Walton DWH (1999) Estimates of structural complexity in clonal plant morphology: comparisons of grazed and ungrazed *Acaena magellanica* rhizomes. *Canadian Journal of Botany*, 77: 869-876.

Price EAC & Hutchings MJ (1992b) Studies of growth in the clonal herb *Glechoma hederacea*. II. The effect of selective defoliation. *Journal of Ecology*, 80: 39-47.

Price EAC & Hutchings MJ (1996) The effects of competition on growth and form in *Glechoma hederacea*. *Oikos*, 75: 279-290.

Price EAC, Gamble R, Williams GG & Marshall C (2002) Seasonal patterns of partitioning and remobilization of ^{14}C in the invasive rhizomatous perennial Japanese knotweed (*Fallopia japonica* (Houtt.) Ronse Decraene). *Evolutionary Ecology*, 15: 347-362.

R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Richards JH, Mueller RJ, & Mott MT (1988) Tillering in tussock grasses in relation to defoliation and apical bud removal. *Annals of Botany*, 62: 173-179.

Sammul M, Kull K & Tamm A (2003) Clonal growth in a species-rich grassland: results of a 20-year fertilization experiment. *Folia Geobotanica*, 38: 1-20.

Sammul M, Kull K, Niitla T & Möls T (2004) A comparison of plant communities on the basis of their clonal growth patterns. *Evolutionary Ecology*, 18: 443-467.

Schmid B & Bazzaz FA (1990) Plasticity in plant size and architecture in rhizome-derived *vs*. seed derived *Solidago* and *Aster*. *Ecology*, 71: 523-535.

Slade AJ & Hutchings MJ (1987a) The effect of nutrient availability on foraging in the clonal herb *Glechoma hederacea*. *Journal of Ecology*, 75: 95-112.

Slade AJ & Hutchings MJ (1987b) The effects of light intensity on foraging in the clonal herb *Glechoma hederacea*. *Journal of Ecology*, 75: 639-650.

Smith AP & Palmer JO (1976) Vegetative reproduction and close packing in a successional plant species. *Nature*, 261: 232-233.

Stoll P, Egli P & Schmid B (1998) Plant foraging and rhizome growth patterns of *Solidago altissima* in response to mowing and fertilizer application. *Journal of Ecology*, 86: 341-354.

Sutherland WJ & Stillman RA (1988) The foraging tactics of plants. Oikos, 52: 239-244.

Suzuki JI & Stuefer JF (1999) On the ecological and evolutionary significance of storage in clonal plants. *Plant Species Biology*, 14: 11-17.

Tuomi J, Nilsson P & Åström M (1994) Plant compensatory responses: bud dormancy as an adaptation to herbivory. *Ecology*, 75: 1429-1436.

van der Meijden E, Wijn M & Verkaar HJ (1988) Defense and regrowth, alternative plant strategies in the struggle against herbivores. *Oikos*, 51: 355-363.

Wang Z, Li L, Han X & Dong M (2004) Do rhizome severing and shoot defoliation affect clonal growth of *Leymus chinensis* at ramet population level? *Acta Oecologica*, 26: 255-260.

Wildová R (2004) Below-ground spatial pattern of rhizomes in a grassland community and its relevance to above-ground spatial pattern. *Plant Ecology*, 174: 319-336

Wildová R, Gough L, Herben T, Hershock C & Goldberg DE (2007a) Architectural and growth traits differ in effects on performance of clonal plants: an analysis using a field-parameterized simulation model. *Oikos*, 116: 836-852.

Wildová, R, Wild J & Herben T (2007b) Fine-scale dynamics of rhizomes in a grassland community. *Ecography*, 30: 264-276.

Conclusion du chapitre 2

Ce chapitre nous a permis d'aboutir à plusieurs conclusions. D'une part, les réponses architecturales dépendent des espèces. Néanmoins, aucun ajustement actif (*i.e.* augmentation de la valeur d'un trait) n'a été observé, la défoliation résultant au mieux dans le maintien, sinon dans la baisse de l'investissement dans le réseau de connexions (*i.e.* longueur et nombre de connexions produites, distances inter-ramets). La plasticité de l'architecture clonale ne dépend pas du type de connexions : les espèces ont pu être groupées sur la base de réponses architecturales similaires et indépendamment de leur caractère stolonifère ou rhizomateux. Ainsi, les deux espèces monopodiales (*T. fragiferum* et *T. repens*) ont subi la plus forte baisse du nombre de connexions, certainement du fait de leur nombre restreint de méristèmes disponibles. A l'inverse, *J. articulatus*, *J. gerardii* (rhizomateuses) et *R. repens* (stolonifères) ont montré des réponses architecturales faibles, voire inexistantes. Des contraintes structurelles (*structural blue-print sensu* Huber *et al.* 1999) gouvernent l'architecture clonale et influencent sa plasticité en réponse à la défoliation. En outre, la performance clonale, indicatrice de la tolérance des espèces étudiées à la défoliation, n'est pas liée à la réponse architecturale. Par conséquent, des architectures contrastées peuvent aboutir à des capacités de tolérance similaires et une diversité d'architectures clonales semble pouvoir s'exprimer en conditions pâturées.

CHAPITRE 3 – REPONSES PHYSIOLOGIQUES DES PLANTES CLONALES A LA DEFOLIATION ET AU PATURAGE.

Introduction du chapitre 3

Le stockage de ressources, principalement carbonées et azotées, est un processus présent chez la majorité des plantes. Cependant, du fait de la production d'organes clonaux et de ramets nombreux, la capacité de stockage est accentuée chez les plantes clonales. En effet, les organes clonaux souterrains tels que les rhizomes, les bulbes ou les tubercules, mais aussi les connexions aériennes, telles que les stolons, ainsi que la base des tiges des ramets peuvent jouer un rôle fondamental dans la mise en place de réserves.

Bien que coûteux pour la plante, le stockage de ressources constitue un mécanisme de tolérance aux stress et aux perturbations. Suite à un évènement de défoliation, la remobilisation rapide des réserves depuis les organes de stockage vers les zones endommagées correspond généralement à la première phase de croissance compensatoire. En présence de bourgeons végétatifs viables, le stockage de carbone permettrait également la régénération végétative. En effet, les substances mises en réserves permettraient de soutenir le développement des bourgeons végétatifs activés par la défoliation notamment du fait de la levée de dominance apicale.

Chez les Poaceae, la base des tiges des ramets contient des stocks importants de réserves, notamment sous la forme de fructanes. Son rôle semble d'autant plus important dans la croissance compensatoire que sa proximité avec les tissus endommagés permettrait une translocation et une remobilisation rapide des réserves. Le stockage de réserves carbonées dans la base des tiges et leur remobilisation rapide suite à la défoliation pourrait être une stratégie avantageuse en prairies pâturées, puisqu'il permettrait une reprise de croissance et/ou une régénération efficace, conférant à l'individu clonal un avantage compétitif sur les plantes voisines.

Après un rapide bilan des connaissances actuelles sur le rôle des réserves dans la tolérance à la défoliation (ARTICLE 6), nous avons testé si le pâturage favorise le stockage de réserves dans la base des tiges de Poaceae (ARTICLE 7). En nous focalisant sur les substances carbonées (sucres), nous avons testé deux hypothèses :

1- La capacité de stockage varie à l'échelle inter-spécifique et dépend du niveau de résistance des espèces au pâturage : les espèces les plus résistantes (dont l'abondance augmente avec le régime de pâturage) stockent des quantités de

réserves plus importantes que les moins résistantes (dont l'abondance diminue quand le régime de pâturage augmente).

2- A l'échelle intra-spécifique, les réserves sont plus abondantes chez les plantes se développant sous un régime de pâturage intense que sous pâturage modéré.

L'ARTICLE 6 est une revue bibliographique. L'étude présentée dans l'ARTICLE 7, a été réalisée sur six espèces clonales pérennes de la famille des Poaceae. Nous avons dosé les sucres non structuraux (amidon, fructanes, saccharose, glucose et fructose) contenus dans la base des tiges de fragments clonaux collectés sous deux régimes de pâturage (modéré et intense).

Article 6 – Storage in clonal plants: a key of their ecological success in disturbed habitats?

Marie-Lise Benot, Anne Bonis & Cendrine Mony

UMR CNRS 6553, ECOBIO, Université de Rennes 1, Campus de Beaulieu, 35042 Rennes Cedex, France

Review article

In preparation

Abstract

Storage, which corresponds to the capacity to save resources, principally carbon and nitrogen, may take place in all plant organs. However organs of clonal multiplication, notably below-ground (rhizomes, bulbs, tubers) or above-ground (stolons) stem-derived structures, as well as shoot bases, may specialize in storage. Storage can occur at several time scales, from hours to months and may vary in function of environmental conditions and, in temperate climates, of seasonal cycles. Yet, long-term storage may also enable the plant to cope with harsh environmental conditions. Large amounts of reserves can buffer stressful conditions, during which growth is slowed down. Storage could also be involved in recovery following disturbance. Although reserve remobilization may interact with current root uptake and photosynthesis, the relative importance of this processes seems to vary with time following damage. Following experimental defoliation, compensatory growth can be divided into two phases. During the very first times after damage (from hours to a few days), refoliation relies mainly on the retranslocation of stored reserves. In grasses, a major part of carbohydrates comes from the stubble, composed of elongating leaf blades enclosed in mature leaf sheaths. During a second phase, root uptake and photosynthesis are restored and current assimilates become the substrate for regrowth. If the plant is not damaged again, pools of reserves can further be replenished. Reserves have also been shown as an important feature of vegetative regeneration following defoliation as they support the development of active buds. In clonal plants, resources and meristems stored in clonal organs and protected from damage could allow ramet production despite disturbances. Consequently, storage appears as a key mechanism of recovery following damage. Particularly developed in clonal plants, this property could partly explain their ecological success in a wide range of ecosystems, notably in disturbed habitats, such as grazing meadows.

Key words

Compensatory growth; defoliation; grazing; remobilization; reserve compounds; resprouting; storage organs.

Introduction

Storage can be considered as the capacity of a plant to save resources, which can be further mobilized to support growth and other functions (Chapin *et al.* 1990). Three kinds of storage can be distinguished: accumulation, reserve making and recycling (Chapin *et al.* 1990). Accumulation refers to an increase in the quantity of compounds that are not immediately needed for growth. Accumulation of nutrients absorbed by roots but not immediately used by the plant is often called luxury uptake or luxury consumption (Lipson *et al.* 1996, Oyarzabal & Oesterheld 2009). Contrary to accumulation, reserve formation corresponds to the synthesis and storage of substances at the expense of current growth, tissue maintenance or defence against attackers. Finally, recycling refers to the degradation of substances that would otherwise be lost as litter to support further growth.

Storage, and particularly reserve formation, represents an immediate cost for the plant, as it diverts resources from potential growth, decreasing growth rate and leading to smaller and less competitive plants (Kobe 1997, van der Meidjen *et al.* 2000, de Jong & van der Meijden 2000). Accumulation may also represent a cost for the plant due to the eventual toxicity, or the negative feedbacks of substances accumulated in great amounts on current photosynthesis and root uptake (Lipson *et al.* 1996, Monson *et al.* 2006). Storage is also expected to involve costly mechanisms such as the production of specialized tissues or the translocation of substances against gradients (Monson *et al.* 2006). Consequently, this property may be advantageous only in environments where its benefits outweigh the immediate costs of reduced growth and toxicity.

In a previous review, Suzuki & Stuefer (1999) highlighted that storage should be selected for in disturbed or temporally fluctuating environments. Indeed, plant individuals are expected to benefit from stored resources when resources available in the environment are not sufficient or cannot be efficiently acquired to support current growth, for instance under resource shortage or after the destruction of resource-acquiring tissues. In such conditions, the remobilization of stored resources could enhance survival probability and allow to maintain growth (Iwasa & Kubo 1997).

Although their relative abundance varies according to environmental conditions, clonal plants are ubiquitous (Klimeš *et al.* 1997) and can notably be found in disturbed habitats (Song & Dong 2002, Klimeš *et al.* 2007, Evette *et al*. 2009). Such ecological success could partly rely on capability of clonal structures to store not only resources but also meristems (Suzuki & Stuefer 1999, Klimešová and Klimeš 2003). The purpose of the present study is to make an inventory of the current knowledge on the relevance of storage in clonal

plants as a strategy to tolerate disturbances. We particularly focus on reserve making. First, we briefly list the major reserve compounds, storage organs and time-patterns of reserve formation, which have already been extensively described (Chapin *et al.* 1990, Suzuki & Stuefer 1999). We then consider studies as evidence for the importance of storage in harsh environmental conditions. After having briefly evocated studies on stressful conditions, we discuss how reserves can enhance compensatory growth and vegetative regeneration after above-ground damage. We finally open the question of the adaptive value of storage in clonal herbaceous species in grazed meadows.

Reserve compounds

Carbon and nitrogen represent the two main compounds stored in plants. Carbon reserves are mainly constituted of carbohydrates and, more particularly, total non-structural carbohydrates (TNC). TNC include polymers such as starch, generally composed of a mixture of two polymers of glucose (amylose and amylopectin, Cairns *et al.* 2002) and fructans (polymers of fructose), dissacharides (sucrose) and monosaccharides, notably hexoses (glucose, fructose). Except starch, which is insoluble in water, the other TNC are often grouped under the term of water-soluble carbohydrates (WSC). According to the species and storage organs, the nature of reserves can change. Although starch is often considered as the principal form of long-term carbon reserves (Manner 1985), this may not always be true. In C3 grasses from temperate regions, starch is the main compound of carbon storage in seeds but not in vegetative tissues, where fructans have often been found in greater amounts (Pollock & Cairns 1991, Scofield *et al.* 2009). Sucrose also represents a significant part of carbohydrates stored in stems of sugar canes and swollen roots of sugar beet, providing them with a crucial economic value (Hawker 1985, John 1992). Some species also store particular carbohydrates, such as the trisaccharides loliose or raffinose (Pavis *et al.* 2001). In addition, amino-acids can constitute a significant source of carbon.

The forms of nitrogen storage seem less well known. Organic nitrogen, in the form of amino-acids and proteins, and nitrates have been proposed as the main forms of nitrogen pools in plants (Millard 1988, Ourry *et al.* 1988, Louahlia *et al.* 1999, Kavanova & Gloser 2005). Lipson *et al.* (1996) demonstrated that luxury uptake of nitrogen was paralleled by the synthesis of nitrogen-rich amino-acids. Soluble proteins have also been shown to be involved in recovery after defoliation in *Lolium perenne*. Moreover, vegetative storage proteins (VSP) have been found in several species, whereas data about grasses are not available, except for *L. perenne*, where they have not been recorded (Louahlia *et al*. 1999, Kavanova & Gloser

2005). Rarer nutrients, such as phosphorus, can also be stored (Chapin 1980, Oyarzabal & Oesterheld 2009).

Storage occurs principally in parenchymatous cells (Kilmes *et al.* 1999), but the cellular location of reserves depends on the substance. Starch is stored in amyloplasts as insoluble granules composed of two polysaccharides, amylose and amylopectin (Manner 1985, Cairns *et al.* 2002). By contrast, despite their polymeric form, fructans are soluble carbohydrates that are stored in vacuoles (Pollock & Cairns 1991). Sucrose represents the major form of carbon translocation and, as such, is usually extra-cellular (Hawker 1985). However, sucrose can participate to carbon storage and, as fructans, can be found in the vacuole. Such a difference between the storage sites of starch and soluble carbohydrates could be a cause of shifts between the types of stored substances. For instance, starch storage could become predominant when the amount of soluble carbohydrates is high, in order to avoid changes in osmotic potential of cells or vacuoles, or negative feedbacks between the concentration of soluble carbohydrates and the synthesis of new assimilates (Scofield *et al.* 2009). Nitrogen reserves compounds are mainly found in the cytoplasm, where amino-acids and proteins are synthesized and in the vacuole. Vacuoles notably contain inorganic forms of nitrogen and phosphorus (Oyarzabal & Oesterheld 2009), while they can specialize in protein storage (protein storage vacuoles, Herman & Larkins 1998).

Storage organs

All plant parts can store resources. However, storage is mainly confined in roots (van der Meijden *et al.* 1988, Kavanova & Gloser 2005), notably tap roots (Tiffin 2000, Meuriot *et al.* 2004), stem bases (Klimeš & Klimešová 2002) and, in the particular case of clonal species, in perennating organs (*e.g.* stolons, rhizomes, tubers, bulbs, Suzuki & Hutchings 1997).

Rhizomes have been suggested to be significantly involved in resource storage (Steen & Larsson 1986, Hartnett 1989, Dong & de Kroon 1994, Suzuki & Stuefer 1999), although this has not always been observed experimentally. In an experiment on *Calamagrostis epigejos*, Kavanova & Gloser (2005) have shown that rhizomes were not a source of nitrogen for the growth of above-ground parts, but that they were rather organs of nutrient translocation from roots. By contrast, rhizomes of *Bistorta bistortoides* proved to provide about 60 % of the nitrogen needed by above-ground parts for their annual growth (Jaeger & Monson 1992). Confirming this observation, Asaeda *et al.* (2006) demonstrated that spring growth of *Phragmites australis* tillers occurred at the expense of pools of TNC stored in rhizomes. In rhizomes, TNC content varies between nodes and internodes. Starch is more

abundant in nodes, probably due to the presence of a developed parenchyma, while soluble carbohydrates rather occur in internodes (Klimeš *et al.* 1999).

Although stolons are often thought to be involved in clonal propagation rather than in storage (Dong & de Kroon 1994), they may contain some amount of reserve substances. For instance, the survival of immature ramets separated from a parent genet of *Potentilla anserina* was enhanced if these ramets remained connected to a stolon fragment (Stuefer & Huber 1999). Internodes of these stolons contained parenchymatous cells, suggesting that they supported young ramets through stored resources.

Moreover, resource storage is particularly important in tillers of grasses and graminoids (Klimeš & Klimešová 2002). In tillers, fructan concentrations can be as high as 30 % of the dry mass and increase according to a gradient from stem apex to its basis (Pollock & Cairns 1991). The bases of vegetative tillers (the stubbles) are composed of new elongating leaves enclosed in the sheaths of mature leaves (Morvan-Bertrand *et al.* 1999b). Water-soluble carbohydrates (WSC), mainly as fructans, can be stored in mature sheath as well as in the elongation zone (*i.e.* blade basis) of immature leaves (de Visser *et al.* 1997, Morvan-Bertrand *et al.* 1999a, b, Scofield *et al.* 2009). In perennial graminoids of seasonal regions, even those with annual tillers senescing in autumn, WSC can persist during winter in the lowest inter-nodes and remaining stubbles of tillers produced by late bud sprouting in autumn (Pollock & Cairns 1991). Such importance of storage in tiller bases may explain the ability of caespitose species, also referred to as consolidative species (de Kroon & Schieving, 1990) to store locally available resources (Cheplick & Chui 2001). This property may be enhanced by the fact that caespitose plants can accumulate carbon and nitrogen, resulting from the degradation of litter, in the 0.1 m of soil below the plant individual (Derner & Briske, 2001).

All clonal structures can fulfil a storage function, suggesting that reserve formation may be of great importance in clonal plants whatever their growth form (*e.g.* rhizomatous, stoloniferous, caespitose, bulb- or tuber-forming). However, although potential, the involvement of clonal organs in resource storage is not always effective (*e.g.* Kavanova & Gloser 2005). On can reasonably expect that it depends at least partly on the conditions the plant encounters.

Timing of reserve formation

Short-term fluctuations

At the day scale, reserve formation is closely related to photosynthesis and takes place principally in the leaves. Starch and vacuolar sucrose are synthesized and stored during the day and degraded and/or redistributed during the night. The efflux of carbohydrates begins preferentially by recent assimilates followed by products of starch hydrolysis (Chapin *et al.* 1990). By contrast, nitrogen is stored in the leaves during the night and reduced during the day (Chapin *et al.* 1990).

Pools of reserves also vary according to environmental events, *e.g.* related to climatic conditions or nutrient availability. Decreased photosynthesis due to cloudy weather is often accompanied by a decrease in quantities of stored carbon. Similarly, reserve making and accumulation are enhanced in response to nutrient pulses, in particular for species growing in resource poor habitats (Chapin *et al.* 1990, de Kroon & Schieving 1990).

Seasonal fluctuations

Resource storage varies along the year (Steen & Larsson 1986, Klimešová & Klimeš 2003). Despite sharp variations according to the species and the geographical region, in seasonal climates, reserve pools generally decrease during the early phases of the growing period, notably to support the outbreak of regrowth following winter. Pools are replenished when growth slows down or during leaf senescence and nutrient recycling (Chapin *et al.* 1990). Consequently, highest pools of reserves have regularly been recorded in late summer, after the new leaves have completed their development (Pollock & Cairns 1991, Beaulieu *et al.* 1997, Kleijn *et al.* 2005, Asaeda *et al.* 2006). For instance, the allocation of carbon to rhizomes of *Fallopia japonica* increased from June to September, was maintained during winter and reallocated to spring growth (Price *et al.* 2002). Kleijn *et al.* (2005) observed a rapid increase of starch content in storage organs of *Veratum album* once plant growth had been completed, while it decreased in early spring and autumn and was relatively stable during winter. Similarly, Asaeda *et al.* (2006) suggested that storage in *Phragmites australis* occurred from June to October. It was weaker during spring, probably because of the diversion of resources to the growth of above-ground parts and sexual reproduction, and in autumn, maybe in relation to metabolic losses (Asaeda *et al.* 2006).

Storage as a mechanism to cope with harsh environmental conditions

Stress tolerance

According to Grime (1977), stress refers to environmental factors reducing the production of plant biomass, for instance resource shortage, oxygen limitation, extreme temperatures or salinity. Reserve making is likely to buffer such conditions and, as such, to be advantageous in stressful environments (Thornton *et al.* 1993). Species with a consolidative strategy are frequent in resource poor conditions, where their ability to rapidly and efficiently uptake available nutrients enable them to survive time lags when nutrients are rare (de Kroon & Schieving 1990). In trees, carbon pools enhance sapling survival and shade tolerance (Kobe 1997). In particular, several studies have suggested the involvement of fructans in stress tolerance. Fructans have been demonstrated to accumulate in response to several abiotic and biotic stresses (Hendry 1987) such as cold temperatures (Chatterton *et al.* 1989), drought (Thomas & James 1999, Amiard *et al.* 2003a), low nutrient availability or fungal infection. Similarly, hypoxia was accompanied by the accumulation of fructans in roots and shoots of several species, regardless their tolerance to flooding, but in a more important manner in flooding-tolerant species (Albrecht *et al.* 1997). Such accumulation is expected to occur because stresses may limit growth while photosynthesis is maintained. Fructans would be advantageous in such conditions of reduced carbon consumption as their accumulation in the vacuole does not prevent photosynthesis and their synthesis is less costly than starch (Albrecht *et al.* 1997). Two major roles of fructans in stress tolerance have been proposed. Fructans could act as osmoregulators buffering stressful conditions, but this function is controversial (Pontis & del Campillo 1985, Hendry 1987). Their hydrolysis after a stress event could also lead to an increase in growth rate once the conditions have become less stressful (Albrecht *et al.* 1993, 1997, Thomas & James 1999).

Disturbance tolerance

Disturbances are generally considered as events resulting in the destruction of part or totality of plant biomass, such as herbivory, fire or wind damage for example (Grime 1977). Mainly considered in the context of herbivory, disturbance tolerance relies on mechanisms enhancing plant regrowth and regeneration following damage (McIntyre *et al.* 1995, 1999, Briske 1996, Strauss & Agrawal 1999). The role of previously stored resources, relative to currently root uptake and photosynthesis, as a mechanism of tolerance to disturbance remains controversial (Tiffin 2000). In general, only a little part of carbon pools is used in response to disturbance

(Chapin *et al.* 1990). Particularly in the case of partial defoliation, after which some photosynthetic tissues are left intact, compensatory growth might preferentially rely on assimilates currently synthesized by remaining photosynthetic tissues, rather than stored resources (Iwasa & Kubo 1997). van der Meijden *et al.* (2000) failed to establish a link between the abilities to store resources and to tolerate herbivory. On the other hand, they noted that plant populations that had experienced long term herbivory (about 20 years) stored larger amounts of resources, suggesting that long-term herbivory had selected for this trait (van der Meijden *et al.* 2000). In fact, several studies have demonstrated that reserves played a key role in recovering from disturbance. Through modelisation, Iwasa and Kubo (1997) affirmed that several disturbance events would decrease pools of stored resources. Such decreases in amounts of reserves have been empirically demonstrated, confirming their involvement in recovery from damages (Klimeš & Klimešová 2002, Kleijn *et al.* 2005, Bråthen & Junttila 2006). Fructans from leaf bases and leaf sheaths are a source of carbon needed to support refoliation (Amiard *et al.* 2003b). In accordance with these observations, phosphorus retranslocation from storage to sink organs has also been shown to enhance regrowth after defoliation (Oyarzabal & Oesterheld 2009).

Indeed, the advantage provided by efficient mobilization of stored resources depends on several factors. The severity of defoliation, as well as the recovery time between consecutive disturbance events, is expected to constrain the efficiency of reserve mobilization (de Jong & van der Meijden 2000, Amiard *et al.* 2003b). For instance, frequent disturbance may prevent sufficient replenishment of reserve pools (de Jong & van der Meijden 2000). The advantage of carbohydrate storage also depends on the presence and activity of meristems. In the absence of active meristems, mobilization of reserves may be unlikely to enhance refoliation (Morvan-Bertrand *et al.* 1999a).

Two phases of compensation after defoliation

Several studies aimed to disentangle the relative role of pre- and post-disturbance resources in regrowth. Most attention has been paid to responses to experimental defoliation, which is considered to mimic above-ground damage caused by main disturbance types, notably herbivory. Two phases of recovery have generally been distinguished. First, the reallocation of previously stored reserves is thought to enable an efficient replacement of lost tissues in the very first times (from hours to about a week) following damage. Second, this phase is relayed by current resource uptake and photosynthesis (Schnyder & de Visser 1999). However, the mobilization of reserves and the assimilation of newly fixed nutrients are not mutually

exclusive and the distinction between these two phases relies on the relative importance of these two sources of nutrients.

The first phase, consisting of short-term mobilization of reserves and reallocation to leaf meristems, is a key process of regrowth after damage (Richards 1993, Lattanzi *et al.* 2004). Indeed, as defoliation partially or totally damages photosynthetic tissues, plants firstly rely on stored resources to recover their photosynthetic activity (de Visser *et al.* 1997, Morvan-Bertrand *et al.* 1999a, b). This early phase of regrowth is characterized by a decline in the biomass of storage organs (Louahlia *et al.* 2000). This transient period lasts from one to a few days (Richards 1993) according to the species and the environmental conditions. Moreover, although clearly described for the remobilization of stored carbohydrates, the existence of this first phase is less clear for nutrients, notably nitrogen.

In experimental studies with *L. perenne*, Morvan-Bertrand *et al.* (1999a, b) observed a decrease of the amount of fructans contained both in leaf sheaths and elongating bases of immature leaves, during about 6 days after defoliation. This decrease of fructan concentration was concomitant with an increase in the fructan exohydrolase (FEH) activity, confirming the role of the products of fructan hydrolysis as a source of carbon for regrowth (Morvan-Bertrand *et al.* 1999b). However, this study showed that, not later than two days after defoliation, a half of carbon supplied to growing zone already originated from newly incorporated carbon. Thus, photosynthetic efficiency was rapidly recovered and was involved in regrowth together with reserve mobilisation.

Some studies have shown an immediate decrease of root activity and nitrogen uptake after defoliation (Louahlia *et al.* 2000, Kavanova & Gloser, 2005). By contrast, Jarvis & Macduff (1989) recorded maintenance of nitrogen absorption for about 15 hours after defoliation and then a decrease for several days. In *L. perenne*, root uptake appeared to be an important source of nitrogen translocated to regrowing tissues readily after defoliation (de Visser *et al.* 1997). In accordance with this observation, Morvan-Bertrand *et al.* (1999a) did not find any relation between the level of nitrogen or soluble proteins contained in the stubble and regrowth efficiency in *L. perenne* during the very first days after defoliation. By contrast, Schnyder & de Visser (1999) distinguished, in the same species, a transient phase of N-remobilisation from reserves during about three days. Consequently, the roles of nitrogen reserves in recovery from defoliation seem contrasted and may depend on the species as well as the growing conditions and severity of defoliation. Indeed, the mobilization of nitrogen reserves and the ongoing root uptake are likely to interfere together during refoliation.

The second phase of regrowth becomes dominant after the restoration of functional photosynthetic tissues. Newly synthesized photo-assimilates become the major source of carbon incorporated in elongating leaves (de Visser *et al.* 1997). Thus, environmental factors influencing photosynthetic rate and nutrient uptake become the major constraints acting on compensatory growth. This time lag also enables the replenishment of reserve pools (Morvan-Bertrand *et al.* 1999b). This process is quite slow and may last several weeks and even months (Richards 1993, Klimeš & Klimešová 2002) but its duration likely depends on the severity of disturbance.

Regeneration following damage

By damaging above-ground tissues, disturbances often release apical dominance and result in the activation of either vegetative or reproductive axillary buds. Such bud sprouting after damage can be considered as a mechanism of tolerance, which enhances regeneration after disturbance (Tuomi *et al.* 1994). The activation of vegetative buds following disturbance, *i.e.* vegetative regeneration, has been particularly studied in woody plants (see for instance Bellingham & Sparrow 2000, Del Tredici 2001, Bond & Midgley 2001, 2003, Lasso *et al.* 2009), while it also takes place in herbaceous species (Klimešová & Klimeš 2003, Latzel *et al.* 2008). Two contrasted strategies have been distinguished: (re)sprouters invest in the constitution of a bud bank, which support vegetative regeneration after damage, whereas seeders rely on seed output to regenerate (Bellingham & Sparrow 2000). Respouting is expected to be advantageous under damage of moderate severity but high frequency (Bellingham & Sparrow 2000, Bond & Midgley 2001).

Vegetative regeneration relies on the presence of bud stored as a bud bank, but it depends also on the pools of stored TNC to support bud growth (Bond & Midgley 2001, Klimešová & Klimeš 2003, Knox & Clarke 2005). Greater amounts of TNC, notably starch, which is the main storage TNC in woody species (Chapin *et al.* 1990, Bell & Ojeda 1999), have been recorded in roots of resprouters than of close relative seeders (Bell & Ojeda 1999, Verdaguer & Ojeda 2002, Knox & Clarke 2005). This enhanced capacity of starch storage is accompanied by larger amounts of specialized root storage tissues (*i.e.* parenchymatic rays, Bell & Ojeda 1999). Where above-ground disturbances cause weaker damages than fire, stems of resprouters can also be involved in starch storage (Nzunda *et al*. 2008).

While only a few sprouters are clonal, almost all of clonal plants have the ability to resprout (Bond & Midgley 2001, 2003). Clonal organs serve as carriers of vegetative buds. Due to their spatial position either below-ground (*e.g.* on rhizomes, bulbs or tubers) or close

to the soil surface (*e.g.* on stolons or bases of grass tillers), they are likely protected from disturbance (Michunas & Noy-Meir 2002, Haukioja & Koricheva 2000, Klimešová & Klimeš 2003, 2007). In clonal plants, resprouting may thus be of great ecological significance as it allows the clonal individual to maintain clonal multiplication even under disturbed conditions.

Conclusions and perspectives: a long-term advantage of storage in grazed meadows

Clonality provides plants with an enhanced capability of resource storage. In clonal plants, reserve mobilization after disturbance may sustain not only the restoration of damaged tissues (compensatory growth) but also ramet production (resprouting). Physiological studies have demonstrated that the retranslocation of a sufficient amount of stored resources to sustain regrowth and/or resprouting lasts for only a few days. Although transient, this phase is nevertheless crucial as it can expectedly provide the plant individual with a greater growth rate and, consequently, an immediate competitive advantage on defoliated neighbours.

As it may slow down current growth, because of diversion of resource or negative feedbacks of accumulation on photosynthesis and resource uptake, storage is costly to the plant in undisturbed habitats. However, these costs are likely outweighed by the benefits of storage in disturbed habitats (Stuefer & Huber 1999), where it can be considered as a bet-hedging strategy. As a consequence, pools of stored resources immediately available after defoliation are expected to be advantageous in disturbed habitats and, notably, to be selected for by grazing (Richards *et al.* 1993, van den Meijden *et al.* 2000). Although commonly assumed, this proposition has rarely been experimentally tested. In particular, the capacity to store resources could be a key of the ecological success of clonal plants. Determining whether storage indeed represents a long-term advantage in temporally variable and disturbed habitats, for instance in grazed meadows, emerges as an exciting prospect linking the areas of physiological and ecological research.

Literature cited

Albrecht G, Kammerer S, Praznik W & Wiedenroth EM (1993) Fructan content of wheat seedlings (*Triticum aestivum* L.) under hypoxia and following re-aeration. *New Phytologist*, 1123: 471-476.

Albrecht G, Biemelt S & Baumgartner S (1997) Accumulation of fructans following oxygen deficiency stress in related plant species with different flooding tolerances. *New Phytologist*, 136: 137-144.

Amiard V, Morvan-Bertrand A, Billard JP, Huault C, Keller F & Prud'homme MP (2003a) Fructans, but not the sucrosyl-galactosides, raffinose and loliose, are affected by drought stress in perennial ryegrass. *Plant Physiology*, 132: 2218-2229.

Amiard V, Morvan-Bertrand A, Billard JP, Huault C & Prud'homme MP (2003b) Fate of fructose supplied to leaf sheaths after defoliation of *Lolium perenne* L.: assessment by ^{13}C-fructose labeling. *Journal of Experimental Botany*, 54: 1231-1243.

Asaeda T, Rajapakse L, Manatunge J & Sahara N (2006) The effect of summer harvesting of *Phragmites australis* on growrth characteristics and rhizome resource storage. *Hydrobiologia*, 553: 327-335.

Beaulieu J, Gauthier G & Rochefort L (1997) The growth response of graminoid plants to goose grazing in a High Arctic environment. *Journal of Ecology*, 84: 905-914.

Bell TL & Ojeda F (1999) Underground starch storage in *Erica* species of the Cape Floristic Region – differences between seeders and resprouters. *New Phytologist*, 144: 143-152.

Bellingham PJ & Sparrow AD (2000) Resprouting as a life history strategy in woody plant communities. *Oikos*, 89: 409-416.

Bond WJ & Midgley JJ (2001) Ecology of sprouting in woody plants: the persistence niche. *Trends in Ecology and Evolution*, 16: 45-51.

Bond WJ & Midgley JJ (2003) The evolutionary ecology of sprouting in woody plants. *International Journal of Plant Science*, 164(Suppl.): S103-S114.

Bråthen KA & Junttila O (2006) Infertile times: response to damage in genets of the clonal sedge *Carex bigelowii*. *Plant Ecology*, 187: 83-95.

Briske DD (1996) Strategies of plant survival in grazed systems: a functional interpretation. In: Hodgson J & Illius AW (eds) *The Ecology and management of grazing systems*. CAB International, Wallingford, pp 33-67.

Cairns AJ, Cookson A, Thomas BJ & Turner LB (2002) Starch metabolism in the fructan-grasses: patterns of starch accumulation in excised leaves of *Lolium temulentum* L. *Journal of Plant Physiology*, 159: 293-305.

Chapin FS III (1980) The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics*, 11: 233-260.

Chapin FS III, Schulze ED & Mooney HA (1990) The ecology and economics of storage in plants. *Annual Review of Ecology and Systematics*, 21: 423-447.

Chatterton NJ, Harrison PA, Bennett JH & Asay KH (1989) Carbohydrate partitioning in 185 accessions of Gramineae grown under warm and cool temperatures. *Journal of Plant Physiology*, 134: 169-179.

Cheplick GP & Chui T (2001) Effect of competitive stress on vegetative growth, storage, and regrowth after defoliation in *Phleum pratense*. *Oikos*, 95: 291-299.

de Jong T & van der Meijden E (2000) On the correlation between allocation to defence and regrowth in plants. *Oikos*, 88: 503-508.

de Kroon H & Schieving F (1990) Resource partitioning in relation to clonal growth strategy. In: van Groenendael J & de Kroon H (eds) *Clonal growth in plants: regulation and function*. SPB Academic Publishing, The Hague, pp 113-130.

de Visser R, Vianden H & Schnyder H (1997) Kinetics and relative significance of remobilized and current C and N incorporation in leaf and root growth zones of *Lolium perenne* after defoliation: assessment by ^{13}C and ^{15}N steady-state labelling. *Plant, Cell and Environment*, 20: 37-46.

Del Tredici P (2001) Sprouting in temperate trees: a morphological and ecological review. *Botanical Review*, 67: 121-140.

Derner JD & Briske DD (2001) Below-ground carbon and nitrogen accumulation in perennial grasses: a comparison of caespitose and rhizomatous growth forms. *Plant and Soil*, 237: 117-127.

Dong M & de Kroon H (1994) Plasticity in morphology and biomass allocation in *Cynodon dactylon*, a grass species forming stolons and rhizomes. *Oikos*, 70: 99-106.

Evette A, Bédécarrats A & Bornette G (2009) Environmental constraints influence clonal traits of herbaceous plant communities in an Alpine Massif. Folia Geobotanica, 44: 95-108.

Grime JP (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist*, 111: 1169-1194.

Hartnett DC (1989) Density- and growth stage-dependent responses to defoliation in two rhizomatous grasses. *Oecologia*, 80: 414-420.

Haukioja E & Koricheva J (2000) Tolerance in woody *vs.* herbaceous plants. *Evolutionary Ecology*, 14: 551-562.

Hawker JS (1985) Sucrose. In: Dey PM & Dixon RA (eds) *Biochemistry of storage carbohydrates in green plants*. Academic Press, London, pp 1-51.

Hendry G (1987) The ecological significance of fructan in a contemporary flora. *New Phytologist*, 106: 201-216.

Herman EM & Larkins BA (1998) Protein storage bodies and vacuoles. *The Plant Cell*, 11: 601-613.

Iwasa Y & Kubo T (1997) Optimal size of storage for recovery after unpredictable disturbances. *Evolutionary Ecology*, 11: 41-65.

Jaeger CH & Monson RK (1992) Adaptive significance of nitrogen storage in *Bistorta bistortoides*, an alpine herb. *Oecologia*, 92: 578-585.

Jarvis SC & Macduff JH (1989) Nitrate nutrition of grasses from steady-state supplies in flowing solution culture following nitrate deprivation and/or defoliation. I. Recovery of uptake and growth and their interactions. *Journal of Experimental Botany*, 218: 965-975.

John P (1992) *Biosynthesis of the major crop products.* John Wiley & Sons, Chichester.

Kavanova M & Gloser V (2005) The use of internal nitrogen stores in the rhizomatous grass *Calamagrostis epigejos* during regrowth after defoliation. *Annals of Botany*, 95: 457-463.

Kleijn D, Treier UA & Müller-Schärer H (2005) The importance of nitrogen and carbohydrate storage for plant growth of the alpine herb *Veratum album*. *New Phytologist*, 166: 565-575.

Klimeš L, Klimešovà J, Hendricks R & van Groenendael J (1997) Clonal plant architecture: a comparative analysis of form and function. In: de Kroon H & van Groenendael J (eds) *The ecology and evolution of clonal plants*. Backhuys Publishers, Leiden, pp 1-29.

Klimeš L, Klimešová J & Čižková H (1999) Carbohydrate storage in rhizomes of *Phragmites australis*: the effect of altitude and rhizome age. *Aquatic Botany*, 64: 105-110.

Klimeš L & Klimešová J (2002) The effects of mowing and fertilization on carbohydrate reserves and regrowth of grasses: do they promote plant coexistence in species-rich meadows? *Evolutionary Ecology*, 15: 363-382.

Klimešová J & Klimeš L (2003) Resprouting of herbs in disturbed habitats: is it adequately described by Bellingham - Sparrow's model? *Oikos*, 103: 225-229.

Klimešovà J & Klimeš L (2007) Bud banks and their role in vegetative regeneration – A literature review and proposal for simple classification assessement. *Perspectives in Plant Ecology, Evolution and Systematics*, 8: 115-129.

Knox KJE & Clarke PJ (2005) Nutrient availability induces contrasting allocation and starch formation in resprouting and obligate seedling shrubs. *Functional Ecology*, 19: 690-698.

Kobe RK (1997) Carbohydrate allocation to storage as a basis of interspecific variation in sapling survivorship and growth. *Oikos*, 226-233.

Lasso E, Engelbrecht BMJ, Dalling JW (2009) When sex is not enough: ecological correlates of resprouting capacity in congeneric tropical forest shrubs. *Oecologia*, 161: 43-56.

Lattanzi FA, Schnyder H & Thornton B (2004) Defoliation effects on carbon and nitrogen substrate import and tissue-bound efflux in leaf growth zones of grasses. *Plant, Cell and Environment*, 27: 347-356.

Latzel V, Mihulka S & Klimešovà J (2008) Plant traits and regeneration of urban plant communities after disturbance: does the bud bank play any role? *Applied Vegetation Science*, 11: 387-394.

Lipson DA, Bowman WD & Monson RK (1996) Luxury uptake and storage of nitrogen in the rhizomatous alpine herb, *Bistorta bistortoides*. *Ecology*, 77: 1277-1285.

Louahlia S, Macduff JH, Ourry A, Humphreys M & Boucaud J (1999) Nitrogen reserve status affects the dynamics of nitrogen remobilization and mineral nitrogen uptake during recovery of contrasting cultivars of *Lolium perenne* from defoliation. *New Phytologist*, 142: 451-462.

Louahlia S, Lainé P, Thornton B & Boucaud J (2000) The role of N-remobilisation and the uptake of NH_4^+ and NO_3^- by *Lolium perenne* L. in laminae growth following defoliation under field conditions. *Plant and Soil*, 220: 175-187.

Manner DJ (1985) Starch. In: Dey PM & Dixon RA (eds) *Biochemistry of storage carbohydrates in green plants*. Academic Press, London, pp 149-203.

McIntyre S, Lavorel S & Tremont RM (1995) Plant life-history attributes: their relationship to disturbance response in herbaceous vegetation. *Journal of Ecology*, 83: 31-44.

McIntyre S, Lavorel S, Landsberg J & Forbes TDA (1999) Disturbance response in vegetation – toward a global perspective on functional traits. *Journal of Vegetation Science*, 10: 621-630.

Meuriot F, Avice JC, Simon JC, Lainé P, Decau ML & Ourry A (2004) Influence of initial organic N reserves and residual leaf area on growth, N uptake, N partitioning and N storage in alfalfa (*Medicago sativa*) during post-cutting regrowth. *Annals of Botany*, 94: 311-321.

Milchunas DG & Noy-Meir I (2002) Grazing refuges, external avoidance of herbivory and plant diversity. *Oikos*, 99: 113-130.

Millard P (1988) The accumulation and storage of nitrogen by herbaceous plants. *Plant, Cell & Environment*, 11: 1-8.

Monson RK, Rosenstiel TN, Forbis TA, Lipson DA & Jaeger CH III (2006) Nitrogen and carbon storage in alpine plants. *Integrative and Comparative Biology*, 46: 35-48.

Morvan-Bertrand A, Boucaud J & Prud'homme MP (1999a) Influence of initial levels of carbohydrates, fructans, nitrogen, and soluble proteins on regrowth of *Lolium perenne* L. cv. Bravo following defoliation. *Journal of Experimental Botany*, 50: 1817-1825.

Morvan-Bertrand A, Pavis N, Boucaud J & Prud'homme MP (1999b) Partitioning of reserve and newly assimilated carbon in roots and leaf tissues of *Lolium perenne* during regrowth after defoliation : assessment by ^{13}C steady-state labelling and carbohydrate analysis. *Plant, Cell and Environment*, 22: 1097-1108.

Nzunda EF, Griffiths ME & Lawes MJ (2008) Sprouting by remobilization of above-ground resources ensures persistence after disturbance of coastal dune forest trees. *Functional Ecology*, 22: 577-582.

Ourry A, Boucaud J & Salette J (1988) Nitrogen mobilization from stubble and roots during re-growth of defoliated perennial ryegrass. *Journal of Experimental Botany*, 39: 803-809.

Oyarzabal M & Oesterheld M (2009) Phosphorus reserves increase grass regrowth after defoliation. *Oecologia*, 159: 717-724.

Pavis N, Chatterton NJ, Harrison PA, Baumgartner S, Praznik W, Boucaud J & Prud'homme MP (2001) Structure of fructans in roots and leaf tissues of *Lolium perenne*. *New Phytologist*, 150: 83-95.

Pollock CJ & Cairns AJ (1991) Fructan metabolism in grasses and cereals. *Annual Review of Plant Physiology and Plant Molecular Biology*, 42: 77-101.

Pontis HG & del Campillo E (1985) Fructans. In: Dey PM & Dixon RA (eds) *Biochemistry of storage carbohydrates in green plants*. Academic Press, London, pp 205-227.

Price EAC, Gamble R, Williams GG & Marshall C (2002) Seasonal patterns of partitioning and remobilization of ^{14}C in the invasive rhizomatous perennial Japanese knotweed (*Fallopia japonica* (Houtt.) Ronse Decraene). *Evolutionary Ecology*, 15: 347-362.

Richards JH (1993) Physiology of plants recovering from defoliation. In: Baker MJ (ed) *Proceedings of the XVII International Grassland Congress*. SIR Publishing, Wellington, pp 85-94.

Schnyder H & de Visser R (1999) Fluxes of reserve-derived and currently assimilated carbon and nitrogen in perennial ryegrass recovering from defoliation. The regrowing tiller and its component functionally distinct zones. *Plant Physiology*, 119: 1423-1435.

Scofield GN, Ruuska SA, Aoki N, Lewis DC, Tabe LM & Jenkins CLD (2009) Starch storage in the stems of wheat plants: localization and temporal changes. *Annals of Botany*, 103: 859-868.

Song M & Dong M (2002) Clonal plants and plant species diversity in wetland ecosystems in China. *Journal of Vegetation Science*, 13: 237-244.

Steen E & Larsson K (1986) Carbohydrates in roots and rhizomes of perennial grasses. *New Phytologist*, 104: 339-346.

Strauss SY & Agrawal AA (1999) The ecology and evolution of plant tolerance to herbivory. *Trends in Ecology and Evolution*, 14: 179-185.

Stuefer JF & Huber H (1999) The role of stolon internodes for ramet survival after clone fragmentation in *Potentilla anserina*. *Ecology Letters*, 2: 135-139.

Suzuki JI & Hutchings MJ (1997) Interactions between shoots in clonal plants and the effect of stored resources on the structure of shoot populations. In: de Kroon H & van Groenendael J (eds) *The ecology and evolution of clonal plants*. Backhuys Publishers, Leiden, pp 311-329.

Suzuki JI & Stuefer JF (1999) On the ecological and evolutionary significance of storage in clonal plants. *Plant Species Biology*, 14: 11-17.

Thomas H & James AR (1999) Partitioning of sugars in *Lolium perenne* (perennial ryegrass) during drought and on rewatering. *New Phytologist* 142: 292-305.

Thornton B, Millard P, Duff EI & Buckland ST (1993) The relative contribution of remobilization and root uptake in supplying nitrogen after defoliation for regrowth of laminae in four grass species. *New Phytologist*, 124: 689-694.

Tiffin P (2000) Mechanisms of tolerance to herbivore damage: what do we know? *Evolutionary Ecology*, 14: 523-536.

Tuomi J, Nilsson P & Åström M (1994) Plant compensatory responses: bud dormancy as an adaptation to herbivory. *Ecology*, 75: 1429-1436.

van der Meijden E, Wijn M & Verkaar H (1988) Defence and regrowth, alternative plant strategies in the struggle against herbivores. *Oikos*, 51: 355-363.

van der Meidjen E, de Boer NJ & van der Veen-van Wijk (2000) Pattern of storage and regrowth in ragwort. *Evolutionary Ecology*, 14: 439-455.

Verdaguer D & Ojeda F (2002) Root starch storage and allocation patterns in seeder and resprouter seedlings of two Cape *Erica* (Ericaceae) species. *American Journal of Botany*, 89: 1189-1196.

Article 7 – Grazing promotes carbohydrate storage in tiller bases

Marie-Lise Benot[1], Annette Morvan-Bertrand[2], Julia Huet[1], Cendrine Mony[1], Cécile Sulmon[1], Marie-Pascale Prud'homme[2] & Anne Bonis[1]

[1]UMR CNRS 6553, ECOBIO, Université de Rennes 1, Campus de Beaulieu, 35042 Rennes Cedex, France

[2]UMR INRA-UCBN 950 EVA Ecophysiologie Végétale, Agronomie et Nutrition NCS, Université de Caen, Esplanade de la Paix, 14032 Caen cedex, France

In prepartation

Abstract

Compensatory growth after defoliation is an essential mechanism of grazing resistance and may be enhanced by the remobilization of stored resources. In grasses, which dominate meadow plant communities, carbohydrates contained in remaining stubbles constitute a major substrate for regrowth after defoliation. The objective of this study was to investigate the role of these carbon pools in grazing resistance. Two hypotheses were tested. We expected carbohydrate reserves to positively related to (i) species resistance to grazing (inter-specific differences) and (ii) regardless the species, to the grazing regime experienced by the plants (intra-specific differences). Six Poaceae species, which differ in their grazing resistance, were selected. Individual plants growing under two contrasted grazing regimes were collected at two sampling dates and carbohydrates contained in the stubbles were measured. Carbohydrate contents were greater just after the grazing season (end of summer) than before the following grazing season (end of winter). Thus, although grazing-induced defoliation may have depleted reserve pools, all species proved able to recover and to replenish these pools between consecutive defoliation events. Fructans and, to a lesser extent, sucrose were the major carbohydrates contained in the stubble of the six study species. At the end of the grazing season, greater amounts of fructose (*i.e.* basic component of fructans) under intensive than under moderate grazing suggested that increasing grazing pressure enhanced fructan hydrolysis and remobilization. At that date, a positive relationship between fructan content and grazing resistance was also detected. Just before the following grazing season, *i.e.* six months after cattle had left the pastures, fructan and sucrose concentrations were not only positively related to grazing resistance but were also higher under intensive than under moderate grazing, regardless the species. Consequently, our results demonstrated that grazing can promote the ability to store carbohydrates, both at the inter- and the intra-specific levels.

Key-words

Carbohydrates, HPLC, grasses, grazing regime, grazing resistance, stubble.

Abbreviations

DW: dry weight, TNC: total non-structural carbohydrates, WSC: water-soluble carbohydrates

Introduction

Grazing induces major changes in floristic and functional compositions in plant communities (Bullock *et al.* 2001, Diaz *et al.* 2001, 2007, de Bello *et al.* 2005), mainly through the consumption of above-ground tissues, *i.e.* defoliation (Kolher *et al.* 2004). Defoliation affects individual plants directly, by removing part of photosynthetically active tissues (leaves and stems) or indirectly, through canopy opening (Sala *et al.* 1986, Richards 1993, Bakker *et al.* 2003, Veen *et al.* 2008). Plant ability to survive and develop after defoliation, *i.e.* compensatory growth (McNaughton 1983, Maschinski & Whitham 1989), is an essential mechanism of grazing tolerance (Briske 1996, Stowe *et al.* 2000). Grazing is thus expected to favor plants able to regrow rapidly after defoliation and to take a competitive advantage over plants missing such ability.

Compensatory growth can be divided into two phases (Richards 1993, Morvan-Bertrand *et al.* 1999b, Schnyder & de Visser 1999). First, defoliation is followed by a transient time lag, during which the mobilization of stored resources is the main mechanism enabling the plant to recover from tissue losses. This period lasts from one day to about a week (Richards 1993, Morvan-Bertrand *et al.* 1999a, b, Schnyder & de Visser 1999). Secondly, photosynthetic activity of newly produced tissues becomes the main source of assimilates, supporting growth and reestablishment of previously consumed reserves (Richards 1993, de Visser *et al.* 1997, Morvan-Bertrand *et al.* 1999b). Consequently, reserves that can be mobilized readily after defoliation could support compensatory growth and enhance short-term competitive ability of recovering plants in grazed areas (Richards 1993).

Storage mainly takes place in roots, perennating organs and stems (van der Meijden *et al.* 1988, Suzuki & Hutchings 1997, Klimeš & Klimešová 2002, Kavanova & Gloser 2005). In Poaceae, great amounts of carbohydrates are stored in the basis of tillers (the *stubble*), which is composed of elongating leaves enclosed in mature leaf sheaths (Ourry *et al.* 1988, Morvan-Bertrand *et al.* 1999b). Carbohydrates contained in remaining stubbles are mobilized rapidly after defoliation, due to their close proximity to the zone of regowth (Morvan-Bertrand *et al.* 2001), and are thus likely to play a key role in compensatory growth.

Carbon is mainly stored as total non-structural carbohydrates (TNC). Starch has long been considered as the most important form of long-term storage TNC (Manner 1985). In Poaceae, starch is the major carbohydrate stored in seeds. However, fructans (*i.e.* polymers of fructose) are the predominant substance of carbon storage in vegetative tissues in most of C3 grasses of temperate areas (Pollock & Cairns 1991), while sucrose or even hexoses (*e.g.* glucose and fructose) can also be involved. No clear relation between amounts of starch and

fructans has been shown and their presence does not seem mutually exclusive (Pontis & del Campillo 1985). The composition of carbohydrate reserves (*i.e.* nature and relative abundances of stored TNS) may change not only according to the species considered, but also to the environmental conditions experienced by the plant (Chatterton *et al.* 1989).

The present study aimed to investigate whether grazing selects for resource storage in the stubble of Poaceae species. In particular, we tested the two following hypotheses.

1- We expected that species should differ in their ability to store resources, depending on their resistance to grazing. Species that are the most resistant to grazing (*i.e.* the abundance of which increases with grazing intensity) should store greater amounts of reserves than species the least resistant to grazing (*i.e.* the abundance of which decreases as grazing intensity increases).

2- Within a species, we expected carbohydrate reserves to be greater in plants developing under intensive grazing than in plants submitted to moderate grazing.

In that purpose, we measured the carbohydrate content (*i.e.* starch, fructans, sucrose, glucose and fructose) in stubbles of individual plants growing in two contrasting grazing regimes for six Poaceae species differing in their grazing resistance.

Material and methods

Study species

This study was carried out on species occurring in the meadow of the Magnils–Reigners (250 ha-large), in the Marais Poitevin (French Atlantic coast, 46° 28'N; 1° 13'W). This grassland has traditionally been grazed by cattle and horses from April to October since the XIIth century. Grazing season thus occurs during spring and summer. An experimental design was established in this meadow and has enabled to control the grazing pressure (from no to intensive grazing) and herbivore type (cattle and/or horses) since 1995 (Amiaud 1998, Loucougary *et al*. 2004, Rossignol *et al*. 2006).

We selected six clonal perennial Poaceae species that commonly occur in this grassland (Benot *et al*. in prep). *Agrostis stolonifera* L., *Cynosurus cristatus* L., *Elytrigia repens* L., *Hordeum secalinum* Schreb., *Lolium perenne* L. and *Poa trivialis* L. are all tussock forming. In addition, *A.stolonifera* can produce stolons and *E. repens* produces long creeping rhizomes. Stolons and rhizomes were not taken into account in the present study. The abundance of these species differs according to the grazing pressure, some of them dominating ungrazed vegetation (*e.g. E. repens*) while others being present only in grazed

conditions (*e.g. L. perenne*). Their resistance to grazing was estimated in Benot *et al*. (in prep.) by (i) estimating their abundance under three contrasting grazing regimes (no, intermediate and intensive cattle grazing pressure) and (ii) carrying out a redundancy analysis on the species abundance matrix, with the grazing regime as the explanatory variable. Species level of grazing resistance corresponded to species score on the constrained axis of the RDA. Low scores corresponded to species abundant without grazing (*i.e.* low grazing resistance). High scores corresponded to species abundant under intensive grazing regime (*i.e.* high grazing resistance; Table 1).

Table 1 – Species score along the constrained axis of the RDA, quantifying species level of grazing resistance (see Benot *et al.* in prep for detailed explanations).

Species	*E.repens*	*A.stolonifera*	*P.trivialis*	*C.cristatus*	*H.secalinum*	*L.perenne*
Score	-0.88	0.20	0.51	0.65	0.67	0.85

Plant collection and material

Non-flowering individuals of each species were collected after a grazing season (October 2008) and two days before the beginning of the following grazing season (April 2009). In October, plants were collected about ten days after the animals had left the pastures and may present traces of leaf damage, indicating former grazing. This date corresponded to direct grazing effects. By contrast, plant individuals collected in April were intact. This date represented long-term grazing effects. Data collection lasted for three days, from 10 am to 4 pm. We paid attention to pick up individuals of all species all day long, in order to maximize the daily variation of carbohydrate content within a species and to minimize differences among species. Collections took place in two paddocks of 1 ha, with stocking rates of 2 and 4 cows.ha^{-1} (*i.e.* about 685 and 1370 kg.ha^{-1}, Ménard *et al.* 2002). These stocking rates respectively correspond to moderate (S2) and intensive grazing (S4). Four of the six study species were present only in grazed paddocks and absent in the absence of grazing. Consequently none plant was picked up in the exclosure without grazing. Individuals were composed of several connected tillers. Each individual was picked up with a knife, carefully washed and immediately frozen in liquid nitrogen. From the field to the lab, the samples were transported in a freezer and kept at -80°C until May 2009, when they were freeze-dried. Freeze-dried plants were dissected in order to separate tiller basis, which corresponded to the stubble composed of mature leaf sheaths enclosed elongated leaves, from the rest of the tiller. As tillers might differ in size, the stubble was cut either under the ligule of the older leaf or at

3 cm above the rooting point for longer stubbles (Fig. 1). These stem bases were reduced into powder.

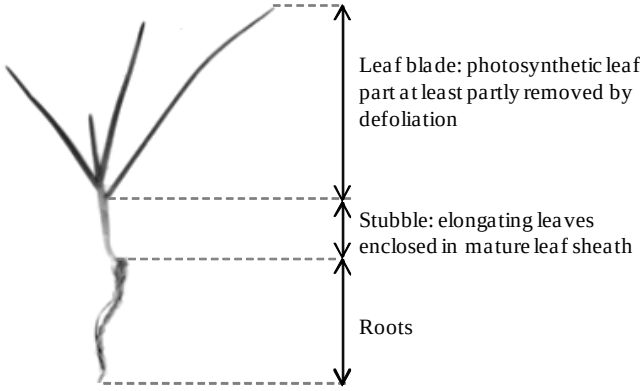

Fig. 1 – View of a tiller of Poaceae and position of the stubble

Extraction, purification and separation of water-soluble carbohydrates (WSC)

Twenty ± 1 mg DW of powder were weighted (the exact weight was noted). WSC (*i.e.* fructans, sucrose, glucose and fructose) were extracted from this powder in 80% ethanol at 80°C for 15 min. After ethanol extraction, the sample was centrifuged at 10,000 *g* for 10 min. The supernatant was preserved and 2 mL of water was added to the pellet. The tube contents were mixed and incubated 15 min at 60 °C. After the first aqueous extraction, the sample was centrifuged at 10,000 *g* for 10 min. The supernatant was preserved and the aqueous extraction was repeated once with the pellet. The three supernatant were pooled, evaporated to dryness under vacuum and the residue was dissolved into 450 µL of ultra pure water. Aliquots of carbohydrate extract (100 µL) were passed through minicolumns (Mobicols from MoBITec, Göttingen, Germany) containing 150 µL of anion exchange resin (Amberlite CG-400 II, formate form, Fluka, Buchs, Switzerland) and 250 µL of cation exchange resin (Dowex 50W X8-400, H+ form, Sigma, Saint-Louis, MO, USA) to remove charged compounds. Between these two resins, 80 µL of PVPP (plyvinylpolypyrrolidone) were added to eliminate lipids, pigments and phenolic compounds.

Glucose, fructose, sucrose, and fructans were separated and quantified by high-performance liquid chromatography (HPLC). The eventual remaining impurities were removed by a pre-column Guard-PAK (Millipore Waters, Milford, MA, USA) and the WSC were then separated on a cation exchange column (Sugar-PAK I, 300 × 6.5 mm, Millipore Waters Milford, MA, USA) eluted at 0.5 $mL.min^{-1}$ with 0.1 mM CaEDTA at 85°C, and detected using a refractometer as a sugar detector.

Starch measurement

Starch was hydrolysed by dissolving the insoluble material remaining after WSC extraction into 200 µL of DMSO (8N) and 50 µL of HCl (8N) at 60°C for 30 min. 200 µL of extract were dissolved into 500 µL of ultra-pure water added with 40 µL of NaOH (5M) and pH was adjusted between 4 and 5. Ultra pure water was added up to 1mL. After decantation, starch content was measured by three successive enzymatic reactions (Enzyplus® kit EZ0 942+ Starch, Raisio Diagnostics SpA, Rome, Italy):

$$\text{Starch} + H_2O + \text{AGS solution (amyloglucosidase)} \rightarrow \text{D-glucose}$$

$$\text{D-glucose} + \text{ATP} + \text{hexokinase} \rightarrow G_6P + \text{ADP}$$

$$G_6P + NADP^+ + G_6PDH \rightarrow 6PG + NADPH + H^+$$

The final product of the reactions (NADPH) was measured by spectrophotometry at 340 nm.

Statistical analyses

The content of carbohydrates was calculated as the carbohydrate mass divided by the dry weight of tissue powder. The impacts of species, grazing regime and their interaction on the concentration of each non-structural carbohydrate were tested through two-way ANOVAs. Tukey HSD tests were applied for post-hoc comparisons. In order to check whether species effects were due to their level of grazing resistance, the factor species was replaced by the variable species score and ANCOVAs were carried out with species score as the covariate, grazing regime as the factor and the interaction of both. When necessary, data were log-transformed for normalisation. The statistical analyses were carried out with the R software (R Development Core Team, 2007, http://www.R-project.org).

Results

Starch

Starch was the least abundant carbohydrate: regarding all species and grazing regimes, starch concentrations in stubbles were lower than 3.5 mg.g DW^{-1} in October and even than 1 mg.g DW^{-1} in April (Fig. 2A, B). In October, starch content depended only on the species (Table 2A). In April, this species effect also occurred but there was a significant impact of the grazing regime (Table 2B): starch content was lower under intensive grazing (S4) than moderate grazing (S2) for *E. repens*, *A. stolonifera* and *H. secalinum*, while no significant difference between both grazing regimes was detected for the three other species (Fig. 2B). At both dates, species effect was not related to species resistance to grazing (Table 3).

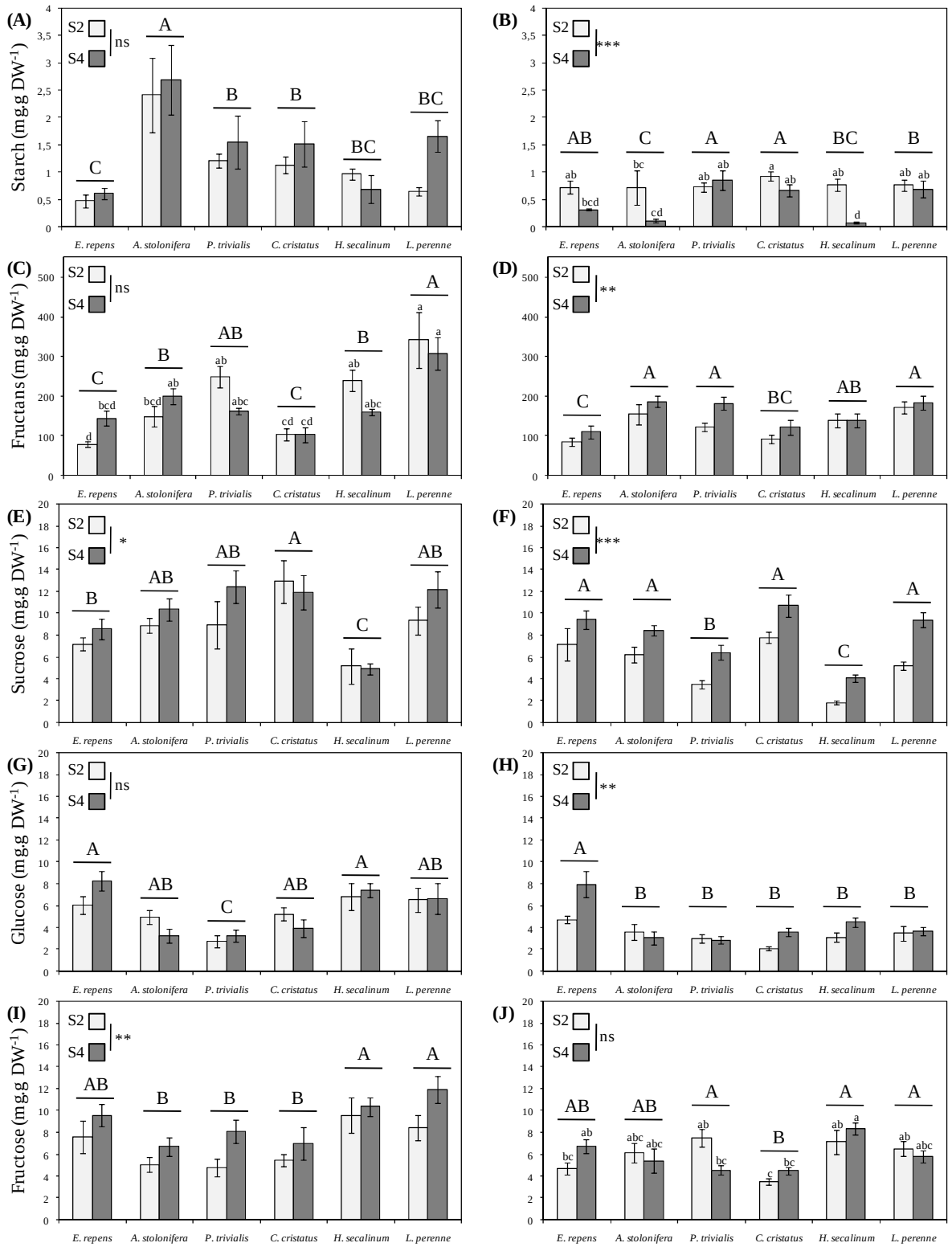

Fig. 2 – Mean (± SE) concentrations (mg.g DW^{-1}) of each of the five non-structural carbohydrates investigated, at both sampling dates (October 2008 and April 2009). Significant differences between the grazing regimes are indicated in the upper right corner of each graph (ANOVA). ns $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Capital letters (A – C) indicate significant differences between the species (ANOVA, $P < 0.05$). Lower case letters (a – d) indicate significant interactions between the species and the grazing regime (Tukey HSD, $P < 0.05$). S2: moderate grazing, S4: intensive grazing.

Fructans

Fructan content in stem bases was largely higher than the content of the other carbohydrates, comprised on average between 80 and 400 mg.g DW^{-1} depending on the species and the date (Fig. 2 – C and D). At both sampling dates, fructan concentration was significantly impacted by the species and the grazing regime (Table 2, Fig. 2C, D). This species effect depended on species resistance to grazing, as indicated by the significant effect of species score on fructan concentration. (Table 4): the higher the species score the greater the amount of fructans (Fig. 2C, D).

In October, the impact of grazing regime on fructan concentration depended on the species (Table 2A). Fructan content was higher under intensive grazing for *E. repens* and *A. stolonifera*, similar between both grazing regimes for *C. cristatus* and *L. perenne* and higher under moderate grazing for *P. trivialis* and *H. secalinum* (Fig. 2C). In April, fructan content was significantly higher for plants submitted to intensive grazing, regardless the species (moderate grazing: 126.5 ± 51.7 mg.g DW^{-1}, intensive grazing: 153.0 ± 54.6 mg.g DW^{-1}; Table 2B, Fig. 2D).

Sucrose

In October 2008, sucrose concentration depended on the species (Table 2A), but independently of their resistance to grazing (Table 3A). This content was the highest for *C. cristatus* and the lowest for *H. secalinum* (Fig. 2E), despite their similar scores in response to grazing (*i.e.* level of grazing resistance, Table 1). Grazing regime also significantly impacted sucrose content (Table 2A), which was higher under intensive grazing, although this difference was weak (moderate grazing: 8.7 ± 4.3 mg.g DW^{-1}, intensive grazing: 10.1 ± 4.3 mg.g DW^{-1}; Fig. 2E). This effect was lost when the species was replaced by species score (Table 3B).

In April 2009, sucrose content was significantly related to both species (Table 2B) and species score (Table 3B), indicating that it tended to be lower for species dominant under intensive grazing (Fig. 2F). Sucrose content was also significantly higher for plants under intensive grazing (moderate grazing: 5.3 ± 2.9 mg.g DW^{-1}, intensive grazing: 8.1 ± 2.9 mg.g DW^{-1}; Table 2B, Fig. 2F).

Table 2 – Results of the ANOVAs testing for the impact of species (Sp.), grazing regime (G.R.) and their interaction on non-structural carbohydrate contents, in October 2008 (A) and April 2009 (B). *P-values* in bold are significant.

		Starch		Fructans		Sucrose		Glucose		Fructose	
	df	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
A – October 2008		n = 94		n = 91		n = 91		n = 91		n = 91	
Sp.	5	9.04	**< 0.001**	17.42	**< 0.001**	11.49	**< 0.001**	8.70	**< 0.001**	5.76	**< 0.001**
G.R.	1	1.83	0.180	0.20	0.659	4.13	0.045	0.11	0.736	11.72	**0.001**
Sp. × G.R.	5	1.51	0.196	3.67	**0.005**	0.72	0.608	1.72	0.139	0.54	0.744
B – April 2009		n = 94		n = 94		n = 94		n = 94		n = 94	
Sp.	5	5.30	**< 0.001**	9.01	**< 0.001**	33.45	**< 0.001**	9.34	**< 0.001**	6.06	**< 0.001**
G.R.	1	21.80	**< 0.001**	8.26	**0.005**	65.53	**< 0.001**	8.72	**0.004**	0.04	0.852
Sp. × G.R.	5	3.09	0.013	0.63	0.677	1.09	0.374	2.09	0.075	3.92	0.003

Table 3 – Results of the ANCOVAs testing for the impact of the species score (Sp. Score), the grazing regime (G.R.) and their interaction on non-structural carbohydrate contents, in October 2008 (A) and April 2009 (B). *P-values* in bold are significant.

		Starch		Fructans		Sucrose		Glucose		Fructose	
	df	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
A – October 2008		n = 94		n = 91		n = 91		n = 91		n = 91	
Sp. score	1	3.47	0.066	17.92	**< 0.001**	0.71	0.402	2.35	0.129	0.12	0.725
G.R.	1	1.05	0.309	0.08	0.779	2.63	0.108	0.22	0.644	8.35	**0.005**
Sp. score × G.R.	1	0.12	0.728	7.28	**0.008**	0.00	0.983	1.17	0.282	0.01	0.913
B – April 2009		n = 94		n = 94		n = 94		n = 94		n = 94	
Sp. score	1	2.01	0.159	10.47	**0.002**	5.89	**0.017**	28.45	**< 0.001**	0.13	0.718
G.R.	1	15.82	**< 0.001**	6.40	**0.013**	22.86	**< 0.001**	7.47	**0.008**	0.02	0.876
Sp. score × G.R.	1	0.45	0.505	0.08	0.779	0.41	0.526	0.76	0.386	2.35	0.129

Hexoses: glucose and fructose

In October, glucose content was only impacted by the species (Table 2A) but independently to the species score (Table 3A). It was the lowest for *P. trivialis* and the highest for both *E. repens* and *H. secalinum* (Fig. 2G). Fructose content was also independent of the species score (Table 3A), but was significantly higher in *H. secalinum* and *L.perenne* than in other species (Fig. 2I). Fructose content was higher for plants in intensive grazing than in moderate grazing regime (moderate grazing: 6.9 ± 3.4 mg.g DW^{-1}, intensive grazing: 9.0 ± 3.5 mg.g DW^{-1}; Fig. 2I).

In April, glucose concentration was significantly higher in *E. repens* than in all other species. It was also significantly, although weakly affected by the grazing regime (moderate grazing: 3.3 ± 1.5 mg.g DW^{-1}, intensive grazing: 4.3 ± 2.1 mg.g DW^{-1}; Table 2B, Fig. 2H). The species impact on fructose content did not depend on species score (Table 3B). Similarly, the response of this hexose to the grazing regime differed according to the species (Table 2B) but independently to their score (Table 3B, Fig. 2J).

Discussion

The effect of grazing on carbohydrate contents in the stubble was estimated at two dates. October sampling (end of summer) was carried out about ten days after the end of the grazing season and April sampling (early spring), two days before the beginning of the following grazing season. Consequently, while October measurements represented direct effects of grazing on carbohydrate contents, April measurements expressed long-term grazing effects, *i.e.* either carry-over effects of the former grazing season or local adaptations to grazing. The amount of carbohydrates was globally higher at the end of summer (October) than after winter (April). In seasonal regions, storage varies throughout the year (Steen & Larsson 1986, Klimešová & Klimeš 2003). Several studies have demonstrated the mobilization of reserves for plant growth in spring, followed by their replenishment during summer, after new foliage has completed its development (Chapin *et al.* 1990, Pollock & Cairns 1991, Beaulieu *et al.* 1997, Kleijn *et al.* 2005, Asaeda *et al.* 2006). However grazing and notably defoliation, which occurred during summer, could have depleted these reserve pools (Beaulieu *et al.* 1997, Klimeš & Klimešová 2002, Kleijn *et al* 2005). Indeed, after defoliation, the mobilization of stored resources to support compensatory growth lasts for about a few days, after which newly produced tissues can resume photosynthesis (Richard 1993).

The greater amounts of carbohydrates recorded at the end of the grazing season indicate that the time lag between two consecutive grazing-induced defoliations may have been long enough to allow not only regrowth but also the replenishment of reserve pools.

Although variable, the ranges of carbohydrate concentrations remained similar amongst the species investigated: fructans were the most abundant non-structural carbohydrate, followed by sucrose, glucose and fructose, while starch was only found as traces. Fructans and starch are known to be more often alternative forms of non-structural carbohydrates (Brocklebank & Hendry 1989), while they can be stored in the same parenchyma cells (Scofield *et al*. 2009). The highly different concentrations of fructans and starch found in the present study confirm the marginal role of starch as a reserve carbohydrate in vegetative tissues for most C3 grasses from temperate climates (Brocklebank & Hendry 1989, Cairns *et al*. 2002). In accordance with this observation, starch appeared little involved in grazing tolerance for the study species. At both sampling dates, the inter-specific variation in starch content in stubbles was not related to species resistance. Moreover starch content sampled just before the beginning of the grazing season (April) was lower, and even close to zero, under intense than under moderate grazing. Given their largely greater abundance in the study species, fructans and sucrose thus emerge as the carbohydrates the most relevant in carbon storage.

Fructan and sucrose concentrations were greater not only for species the most resistant to grazing (*i.e.* the abundance of which increases along a grazing gradient), but also for plants developing under intensive grazing, regardless the species. Our results thus confirmed the hypothesis that carbohydrate storage in the stubble of Poaceae could be favored in grazed vegetation, both at the intra and inter-specific levels. A significant species effect was detected for all of the carbohydrates and both sampling dates. This result indicates that the composition and concentration of the carbohydrate pools in the stubble were primarily constrained by the species, despite their phylogenetic proximity. At both sampling dates, fructan content significantly differed according to the species and was even positively related to species response to grazing. A similar trend occurred for sucrose, but only in April. As a consequence, and contrary to van der Meijden *et al*. (2000), who found no link between storage and grazing tolerance, our results indicate that the content of some carbohydrates, notably fructans and sucrose, was positively related to species resistance to grazing. Similarly, Klimeš & Klimešová (2002) demonstrated, for three Poaceae species, that recovery following mowing was positively related to carbohydrate concentration in stem bases. This tendency was even stronger when absolute quantities, rather than concentrations, were considered. Our

observations may thus have been quite different if we had measured carbohydrate absolute contents. The concentrations that we monitored in tiller bases likely represented the major part of carbohydrates contained in the tiller. Indeed, fructan concentration in stems increases along a gradient from the apex to the base (Pollock & Cairns 1991). A similar trend can be expected for the other, less abundant, carbohydrates. It can thus be concluded that grazing tended to favor species that are the most efficient in fructan and sucrose storage. An effect of grazing, at least as important, was to promote intra-specific variation in the amounts of carbohydrate reserves.

At the end of the grazing season (October 2008), the effect of the grazing regime (*i.e.* moderate *vs.* intense) on fructan content depended on the species resistance to grazing. In species the most resistant to grazing (*C. cristatus, L. perenne, H. secalinum* and *P. trivialis*), intense grazing led either to a maintained or a decreased fructan concentration compared to moderate grazing. By contrast, in the less resistant *E. repens* and *A. stolonifera*, fructan concentration was greater under intense than under moderate grazing regime. At first glance, these observations may indicate a differential use of fructan pools amongst species, during the grazing season. The yield of regrowth is influenced by the efficiency of reserve retranslocation readily after defoliation (Schnyder & de Visser 1999). Fewer or similar amounts of carbohydrates remaining in stubbles under intensive grazing compared to moderate grazing could be related to their efficient remobilization and explain differences in species resistance to grazing. Yet, at the end of the grazing season, fructose contents were greater under intensive than moderate grazing, regardless the species. This suggests that fructan hydrolysis and remobilization as a simple sugar readily available for regrowth may be most important in response to intensive grazing, and such, for all species. Moreover, amounts of fructans accumulated at the end of summer were positively related to species resistance to grazing. Consequently, grazing may favor species on the basis of their ability to make fructan reserves despite defoliation rather than to remobilize these reserves after defoliation.

As expected, grazing impacted the pools of reserves available just before the grazing season (April 2009). Amounts of fructans and sucrose were larger for plants from the intensively grazed paddock than for plants from the moderately grazed one, regardless the species. These observations indicate that grazing impact on the most important storage compounds expressed six months after cattle had left the pastures. Consequently, grazing appeared to favor not only the ability to synthesize large amounts of fructans during summer but also the ability to keep a sufficient pool of reserves available at the beginning of the

following grazing season. These latter are expectedly of great importance as they could promote compensatory growth after defoliation (Morvan-Bertrand *et al*. 1999a).

Our results strongly indicate that fructans and sucrose are involved in grazing tolerance both at the intra- and inter-specific levels. On the contrary, starch had a weak relevance in vegetative storage for all of the six study species. Carbohydrate storage and remobilization readily after defoliation thus appeared advantageous under grazing conditions.

Literature cited

Amiaud B (1998) *Dynamique végétale d'un écosystème prairial soumis à différentes modalités de pâturage. Exemple des communaux du Marais Poitevin*. Thèse de 3ème cycle, Université de Rennes 1.

Asaeda T, Rajapakse L, Manatunge J & Sahara N (2006) The effect of summer harvesting of *Phragmites australis* on growrth characteristics and rhizome resource storage. *Hydrobiologia*, 553: 327-335.

Bakker C, Blair JM & Knapp AK (2003) Does resource availability, resource heterogeneity or species turnover mediate changes in plant species richness in grazed grasslands? *Oecologia*, 137: 385-391.

Beaulieu J, Gauthier G & Rochefort L (1997) The growth response of graminoid plants to goose grazing in a High Arctic environment. *Journal of Ecology*, 84: 905-914.

Benot ML, Mony C, Lepš J & Bonis A (in prep.) Are clonal traits an plastic responses to defoliation good indicators of grazing resistance? A test on eight clonal species (ARTICLE 3).

Briske DD (1996) Strategies of plant survival in grazed systems: a functional interpretation. In: Hodgson J & Illius AW (eds) *The Ecology and management of grazing systems*. CAB International, Wallingford, pp 33-67.

Brocklebank KJ & Hendry JAF (1989) Characteristics of plant species which store different types of reserve carbohydrates. *New Phytologist*, 112: 255-263.

Bullock JM, Franklin J, Stevenson MJ, Silvertown J, Coulson SJ, Gregory SJ & Softs R (2001) A plant trait analysis of responses to grazing in a long-term experiment. *Journal of Applied Ecology*, 38: 253-267.

Cairns AJ, Cookson A, Thomas BJ & Turner LB (2002) Starch metabolism in the fructan-grasses: patterns of starch accumulation in excised leaves of *Lolium temulentum* L. *Journal of Plant Physiology*, 159: 293-305.

Chapin FS III, Schulze ED & Mooney HA (1990) The ecology and economics of storage in plants. *Annual Review of Ecology and Systematics*, 21: 423-447.

Chatterton NJ, Harrisson PA, Bennett JH, Asay KH (1989) Carbohydrate portioning in 185 accessions of Gramineae grown under warm and cool temperatures. *Journal of Plant Physiology*. 134: 169-179.

de Bello F, Lepš J & Sebastià MT (2005) Predictive value of plant traits to grazing along a climatic gradient in the Mediterranean. *Journal of Applied Ecology* 42: 824-833.

de Visser R, Vianden H & Schnyder H (1997) Kinetics and relative significance of remobilized and current C and N incorporation in leaf and root growth zones of *Lolium perenne* after defoliation: assessment by ^{13}C and ^{15}N steady-state labelling. *Plant, Cell and Environment*, 20: 37-46.

Diaz S, Noy-Meir I & Cabido M (2001) Can grazing response of herbaceous plants be predicted from simple vegetative traits? *Journal of Applied Ecology*, 38: 497-508.

Diaz S, Lavorel S, McIntyre S, Falczuk V, Casanoves F, Milchunas DG, Skarpe C, Rusch G, Sternberg M, Noy-Meir I, Landsberg J, Zhang W, Clark H & Campbell BD (2007) Plant trait response to grazing – a global synthesis. *Global Change Biology*, 13: 313-341.

Kavanova M & Gloser V (2005) The use of internal nitrogen stores in the rhizomatous grass *Calamagrostis epigejos* during regrowth after defoliation. *Annals of Botany*, 95: 457-463.

Kleijn D, Treier UA & Müller-Schärer H (2005) The importance of nitrogen and carbohydrate storage for plant growth of the alpine herb *Veratum album*. *New Phytologist*, 166: 565-575.

Klimeš L & Klimešová J (2002) The effects of mowing and fertilization on carbohydrate reserves and regrowth of grasses: do they promote plant coexistence in species-rich meadows? *Evolutionary Ecology*, 15: 363-382.

Klimešová J & Klimeš L (2003) Resprouting of herbs in disturbed habitats: is it adequately described by Bellingham - Sparrow's model? *Oikos*, 103: 225-229.

Kohler F, Gillet F, Gobat JM & Buttler A (2004) Seasonal vegetation changes in mountain pastures due to simulated effects of cattle grazing. *Journal of Vegetation Science*, 15: 143-150.

Loucougaray G, Bonis A & Bouzillé JB (2004) Effects of grazing by horses and/or cattle on the diversity of coastal grasslands in western France. *Biological Conservation*, 116: 59-71

Manner DJ (1985) Starch. In: Dey PM & Dixon RA (eds) *Biochemistry of storage carbohydrates in green plants*. Academic Press, London, pp 149-203.

Maschinski J, Whitham TG (1989) The continuum of plant responses to herbivory: the influence of plant association, nutrient availability, and timing. *American Naturalist*, 134: 1-19.

McNaughton SJ (1983) Compensatory plant growth as a response to herbivory. *Oikos*, 40: 329-336.

Ménard C, Ducan P, Fleurance G, George JY & Lila M (2002) Comparative foraging and nutrition in horses and cattle in Europe wetlands. *Journal of Applied Ecology*, 38: 120-133.

Morvan-Bertrand A, Boucaud J & Prud'homme MP (1999a) Influence of initial levels of carbohydrates, fructans, nitrogen, and soluble proteins on regrowth of *Lolium perenne* L. cv. Bravo following defoliation. *Journal of Experimental Botany*, 50: 1817-1825.

Morvan-Bertrand A, Pavis N, Boucaud J & Prud'homme MP (1999b) Partitioning of reserve and newly assimilated carbon in roots and leaf tissues of *Lolium perenne* during regrowth after defoliation : assessment by ^{13}C steady-state labelling and carbohydrate analysis. *Plant, Cell and Environment*, 22: 1097-1108.

Morvan-Bertrand A, Boucaud J, Le Saos J & Prud'homme MP (2001) Roles of fructans from leaf sheaths and from the elongating leaf bases in the regrowth following defoliation of *Lolium perenne* L. *Planta*, 213: 109-120.

Ourry A, Boucaud J & Salette J (1988) Nitrogen mobilization from stubble and roots during re-growth of defoliated perennial ryegrass. *Journal of Experimental Botany*, 39: 803-809.

Pollock CJ & Cairns AJ (1991) Fructan metabolism in grasses and cereals. *Annual Review of Plant Physiology and Plant Molecular Biology*, 42: 77-101.

Pontis HG & del Campillo E (1985) Fructans. In: Dey PM & Dixon RA (eds) *Biochemistry of storage carbohydrates in green plants*. Academic Press, London, pp 205-227.

R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Richards JH (1993) Physiology of plants recovering from defoliation. In: Baker MJ (ed) *Proceedings of the XVII International Grassland Congress*. SIR Publishing, Wellington, pp 85-94.

Rossignol N, Bonis A & Bouzillé JB (2006) Relationships between grazing pattern and vegetation structure on the spatial variations of net N mineralisation in a wet grassland. *Applied Soil Ecology*, 31: 62-72

Sala OE, Oesterheld M, León RJC & Soriano A (1986) Grazing effects upon plant community structure in subhumid grasslands of Argentina. *Vegetatio*, 67: 27-32.

Schnyder H & de Visser R (1999) Fluxes of reserve-derived and currently assimilated carbon and nitrogen in perennial ryegrass recovering from defoliation. The regrowing tiller and its component functionally distinct zones. *Plant Physiology*, 119: 1423-1435.

Scofield GN, Ruuska SA, Aoki N, Lewis DC, Tabe LM & Jenkins CLD (2009) Starch storage in the stems of wheat plants: localization and temporal changes. *Annals of Botany*, 103: 859-868.

Steen E & Larsson K (1986) Carbohydrates in roots and rhizomes of perennial grasses. *New Phytologist*, 104: 339-346.

Stowe KA, Marquis RJ, Hochwender CG & Simms EL (2000) The evolutionary ecology of tolerance to consumer damage. *Annual Review of Ecology and Systematics*, 31: 565-595.

Suzuki JI & Hutchings MJ (1997) Interactions between shoots in clonal plants and the effect of stored resources on the structure of shoot populations. In: de Kroon H & van Groenendael J (eds) *The ecology and evolution of clonal plants*. Backhuys Publishers, Leiden, pp 311-329.

van der Meijden E, Wijn M & Verkaar H (1988) Defence and regrowth, alternative plant strategies in the struggle against herbivores. *Oikos*, 51: 355-363.

van der Meidjen E, de Boer NJ & van der Veen-van Wijk (2000) Pattern of storage and regrowth in ragwort. *Evolutionary Ecology*, 14: 439-455.

Veen GF, Blair JM, Smith MD & Collins SL (2008) Influence of grazing and fire frequency on small-scale plant community structure and resource variability in native tallgrass prairie. *Oikos*, 117: 859-866.

Conclusion du chapitre 3

L'étude présentée dans l'article 7 confirme le rôle prépondérant des fructanes comme sucre de réserve dans les tissus végétatifs des six espèces de Poaceae étudiées. L'amidon n'est présent que sous forme de traces tandis que le saccharose et les hexoses (glucose et fructose) présentent des concentrations intermédiaires. Nos résultats mettent en évidence l'impact du pâturage sur les réserves carbonées contenues dans la base des tiges aussi bien à l'échelle inter- qu'à l'échelle intra-spécifique.

Les stocks de sucres plus importants en fin de saison de pâturage (octobre 2008) qu'en début de saison de pâturage (avril 2009) indiquent que la synthèse et le stockage de réserves pendant l'été ont eu lieu malgré le pâturage. Les teneurs en sucres sont également dépendantes de l'espèce. Plus particulièrement, les stocks de fructanes et de saccharose disponibles avant la saison de pâturage sont positivement liés à la résistance des espèces. Une différence intra-spécifique a également été détectée. Toutes espèces confondues, les stocks de fructanes et de saccharose disponibles avant la saison de pâturage sont plus importants chez les plantes soumises au pâturage intense que chez les plantes soumises au pâturage modéré.

Chez ces six espèces, le stockage de réserves carbonées dans la base des tiges apparaît donc comme une stratégie favorisée par le pâturage de manière inter- et intra-spécifique.

Les substances de réserves contenues dans les rhizomes des espèces qui en produisent ont également été dosées, mais ce jeu de données est en cours d'analyse et sera seulement brièvement évoqué en conclusion de ce manuscrit.

CHAPITRE 4 – IMPORTANCE RELATIVE DES TRAITS ARCHITECTURAUX ET PHYSIOLOGIQUES DANS LA REPONSE A LA DEFOLIATION. (APPROCHE PAR MODÉLISATION)

Introduction du chapitre 4

Les études présentées au cours des chapitres précédents ont permis de caractériser l'impact du pâturage et de la défoliation sur les traits clonaux.

Nous avons observé que le pâturage bovin intensif tend à homogénéiser la hauteur de la végétation à échelle fine comme à échelle grossière. A l'inverse, un pâturage modéré est plutôt générateur d'hétérogénéité spatiale, notamment à large échelle. Néanmoins, quel que soit le régime de pâturage, la défoliation ne semble pas pouvoir être perçue comme hétérogène à l'échelle du fragment clonal. La sévérité de la défoliation s'appliquant aux fragments clonaux varierait donc surtout en fréquence et en intensité.

Nos résultats empiriques ont également mis en évidence l'existence d'une diversité de réponses architecturales à la défoliation, indépendamment des capacités de tolérance à la défoliation et au pâturage. A l'inverse, le pâturage semble favoriser les capacités de stockage dans la base des tiges des ramets, permettant la tolérance de la défoliation (compensation et/ou régénération suite à la défoliation). Il semblerait donc que les propriétés clonales étudiées (architecture clonale et stockage de ressources) et les traits sous-jacents n'aient pas la même implication dans la réponse au pâturage et à la défoliation.

Toutefois, les mesures *in situ* ne permettent pas de dissocier les divers paramètres de la défoliation ou de les distinguer d'autres facteurs environnementaux, tandis que les approches expérimentales ne permettent généralement que d'en tester quelques uns. Ainsi, lors des expérimentations, nous avons appliqué des traitements de défoliation homogène, différant soit en fréquence soit en intensité (hauteur de coupe). Il est donc difficile d'analyser l'importance relative des divers paramètres de la défoliation sur l'expression des traits clonaux.

En outre, les réponses observées *in situ* ou expérimentalement sont susceptibles de traduire des contraintes développementales ou des corrélations génétiques entre traits, au delà d'une réponse strictement restreinte aux conditions environnementales. Par exemple, les expériences réalisées sur l'architecture clonale ont montré que les contraintes structurales peuvent limiter la plasticité phénotypique en réponse à la défoliation.

Par une approche de modélisation, nous avons donc cherché à caractériser les combinaisons de traits clonaux optimales (*i.e.* qui permettent de maximiser la performance du fragment

clonal) en les associant aux caractéristiques de la défoliation (pourcentage et grain, fréquence, intensité). Plus particulièrement, nous avons testé deux hypothèses :

1- Quelles que soient les caractéristiques de la défoliation, il existe au moins une combinaison de traits clonaux optimale qui permet de maximiser la performance clonale.
2- Les traits clonaux impliqués dans la réponse à la défoliation dépendent de ses caractéristiques. Par conséquent, les combinaisons de traits clonaux optimales diffèrent en fonction des caractéristiques de la défoliation, avec deux sous-hypothèses :
    a. la défoliation hétérogène à l'échelle du genet favorisent l'élongation des connexions et l'intégration physiologique extensive
    b. des fréquences et intensités de défoliation croissantes favorise l'intégration physiologique extensive et la mise en place de réserves.

Nous avons choisi d'aborder ces questions grâce à la modélisation numérique. En effet, celle-ci présente plusieurs avantages par rapport aux expérimentations et mesures de traits *in situ*. Elle permet (i) de tester un grand nombre de patrons de défoliation, décrits sur la base de leurs caractéristiques spatiales (pourcentage et grain de la défoliation), de leur fréquence et de leur intensité, (ii) de s'affranchir des corrélations entre traits et, par conséquent, de déterminer l'importance relative de chaque trait, indépendamment des autres, dans la réponse aux divers patrons de défoliation.

Article 8 – Role of clonal traits in genet response to contrasted patterns of defoliation. A modeling approach.

Marie-Lise Benot[1], Marc Garbey[2], Malek Smaoui[2], Anne Bonis[1] & Cendrine Mony[1]

[1]UMR CNRS 6553, ECOBIO, Université de Rennes 1, Campus de Beaulieu, 35042 Rennes Cedex, France

[2]Department of Computer Science, University of Houston, 501 Philipp G-Hoffman Hall, Houston TX, 77204-3010, USA

In preparation

Abstract

Defoliation is a common disturbance experienced by a wide range of plant communities, most of which are dominated by clonal plants. Defoliation patterns can vary in spatial arrangement, frequency or intensity, and may promote clonal traits maximizing genet performance. The present study aimed to disentangle the relationships between clonal traits and defoliation parameters. Two hypotheses were tested. (1) Amongst the great diversity of clonal growth forms, there exists at least one optimal combination of clonal traits that maximizes genet performance. (2) Optimal combinations of clonal traits differ according to defoliation parameters. More precisely, heterogeneous defoliation should promote both high lateral expansion and extensive physiological integration, while homogeneous but frequent and/or intensive defoliation should favor compact architecture and resource storage. These issues were addressed through an individual-based model simulating the growth of single genets submitted to various defoliation patterns. Three indicators of genet performance were investigated (*i.e.* genet biomass, number of ramets and length of the longest connection). Only biomass was affected by defoliation, notably defoliation percentage, suggesting that defoliation patterns had little impact on genet performance. Yet, the expression of clonal traits varied according to defoliation patterns. Homogeneous defoliation was a strong filter on clonal traits, selecting for only a few combinations. Although the absence of defoliation appeared as a weaker filter, it selected for similar clonal traits. In accordance with our expectations, heterogeneous defoliation selected for high inter-ramet distances leading to dispersed populations of ramets, which enable to spread the risks of defoliation amongst these ones. By contrast, homogeneous conditions favored a potentially high number of primary connections and small inter-ramet distances, leading to an efficient occupation of the ground. Contrary to our expectations, resource storage had no importance in the response to defoliation, regardless of its pattern. When genet performance was estimated by genet biomass, extensive resource sharing was promoted under intermediate defoliation percentage and fine grain. However, the distance of resource sharing became irrelevant to defoliation patterns when the other indicators of performance were investigated. This modeling approach has enabled to analyze the relationship between an important set of clonal traits and defoliation patterns. Further improvements could be implemented, notably by expressing genet performance through a synthetic indicator or including competition with other genets.

Key-words Clonal architecture, clonal fragment, clonal integration, defoliation frequency, defoliation intensity, individual-based model (IBM), spatial heterogeneity, storage.

Introduction

The partial removal of plant above-ground tissues (*i.e.* defoliation), affects a wide range of terrestrial as well as aquatic plant communities (Huntly 1991). It can occur under diverse forms going from herbivory by small invertebrates to grazing by large mammalian herbivores (Huntly 1991) and even human mowing. The proportion of tissue removed (defoliation intensity) or the time lag between consecutive defoliation events (defoliation frequency) may vary, depending on the type, selectivity, size and abundance of herbivores (Huntly 1991, Olff & Ritchie 1998). Defoliation also generates spatial heterogeneity at several scales from plant parts to a whole plant community (Adler *et al*. 2001). These factors modulate the specific diversity and composition of plant communities mainly through their impacts on plant growth and reproduction (Olff & Ritchie 1998, Bullock *et al.* 2001, García & Ehrlén 2002).

Temperate ecosystems are dominated by clonal plants, which represent up to 70 % of vascular plant species (van Groenendael & de Kroon 1990, Klimeš *et al*. 1997). Clonality provides plant individuals (*genets*) with the ability to produce potentially autonomous descendents (*ramets*), usually linked together by plagiotropic connective stems (*connections*) either above-ground or below-ground (*stolons* or *rhizomes*, respectively). Clonal plants are abundant in several types of ecosystems (Klimeš *et al*. 1997). This ecological success possibly relies on the great diversity of clonal growth forms. Indeed environmental conditions, such as contrasted defoliation patterns, may filter clonal traits and promote clonal growth forms succeeding the best (Diaz *et al*. 1998).

The expression of clonal traits gives rise to two major clonal properties. Clonal architecture influences the lateral expansion of the genet and the spatial position of its ramets (Herben & Suzuki 2002). Clonal integration (*i.e*. the physical and physiological integration between ramets) enables resource storage within and resource translocation throughout connections (Suzuki & Stuefer 1999, Oborny *et al.* 2001). These properties govern the ability of a genet to perceive and respond to its environment. Their ecological advantages depend on the environmental conditions, notably spatio-temporal heterogeneity (Hutchings 1999).

Clonal architecture varies along a gradient confined between two extremes. Guerrilla growth forms (*sensu* Lovett-Doust 1981) are characterized by long connections and long fragments of connection linking two consecutive ramets (*spacers sensu* Bell 1984). By contrast, phalanx growth forms (*sensu* Lovett-Doust 1981) invest in connection branching, rather than elongation. The advantages provided by both architectures may depend on the environment (Slade & Hutching 1987a, b, de Kroon & Schieving 1990). On the one hand, an important investment in lateral expansion through random elongation of connections enhances

the area explored by the genet. For instance, guerrilla growth forms lead to an efficient exploration of heterogeneous habitats, as demonstrated either empirically or through model studies (Sutherland & Stillman 1988, de Kroon & Hutchings 1995, Kleijn & van Groenendael 1999). In particular, long connections and spacers may enable the genet to escape from unfavorable conditions (de Kroon & Schieving 1990, Macek & Lepš 2003, Puijalon *et al*. 2008). Consequently, a dispersed position of ramets could be advantageous under heterogeneous defoliation as it would spread the risk of being defoliated among ramets. On the other end, phalanx-type compact architecture maximizes local occupation rather than exploration of space and appears advantageous under homogeneous environments (de Kroon & Shieving 1990).

Resource storage and translocation have regularly been suggested to buffer both spatial and temporal heterogeneity. On the one hand, resources stored in the connections could enable a whole genet to cope with resource shortage (de Kroon & Schieving 1990) and disturbances (Iwasa & Kubo 1997, Kleijn *et al*. 2005, Asaeda *et al*. 2006) that occur under frequent and/or intense defoliation. On the other hand, in spatially heterogeneous environments, long distance resource sharing among ramets (extensive physiological integration) allows source ramets growing in favorable conditions to support sink ramets positioned in unfavorable conditions (Jónsdóttir & Callaghan 1989, Hutchings 1999). Consequently, it is expected to enable a whole genet to average spatial heterogeneity (Alpert 1991, Oborny *et al*. 2000), in particular heterogeneous defoliation of its ramets (Harnett 1989).

The present study aimed to analyze the relationships between the spatio-temporal patterns of defoliation and clonal traits by the means of a modeling study. Contrary to experimental approaches, which are limited by methodological and biological constraints, simulation studies enable to investigate a wide range of clonal forms and defoliation patterns. We tested the two following hypotheses.

1- The patterns of defoliation should not influence clonal plant performance. We assumed that at least one clonal growth form (*i.e.* combination of clonal traits) enables to maximize performance whatever the characteristics of defoliation.

2- Clonal traits maximizing genet performance should differ according to the characteristics of defoliation considered. We assumed that (i) increasing heterogeneity perceptible at the genet scale should promote elongation over branching of the connections as well as extensive integration and (ii) increasing defoliation intensity and frequency should favor resource storage and translocation.

To address these issues we used the individual-based model CLONAL developed by Mony *et al*. (submitted). We simulated the growth of clonal plants submitted to a wide range of defoliation characteristics. At the end of the simulations, we monitored genet performance estimated either by the total biomass, number of ramets produced or lateral expansion.

Material & Methods

Model description

The model is an individual-based model composed of two layers. The *genet layer* corresponds to the model CLONAL described in Smaoui *et al*. (2008) and Mony *et al*. (submitted), which was used to model the clonal expansion of a single clonal plant. In the present study, the second layer (*defoliation pattern layer*) is superimposed to the genet layer and describes the pattern of defoliation applied to the plant. In both layers, space is represented as a 99 × 99 cell hexagonal lattice. While no unit is explicitly specified, one cell corresponds to about 0.02 m.

The genet layer

A genet is modeled as a set of ramets and connective stems. Each ramet corresponds to one cell (called *ramet growth unit*), whereas connections are composed of several cells (called *connection growth units*). A spacer is composed of a variable number of connection growth units, depending on the inter-ramet distance (see Appendix A). A connection is characterized by its generation (*i.e.* order). Connections growing from the parent ramet are called *primary connections* (first generation) and connection branching from other ramets are called *branch connections* (either of second or third generation). The properties of growth units depend on their status: ramet growth units contribute to resource acquisition, while connection growth units are specialized in resource translocation and storage.

The growth of the genet is governed by processes, which are described by a set of probabilistic laws. A detailed description of the growth rules is provided in Appendix A. These rules rely on 16 input parameters corresponding to (i) rules of resource acquisition and basic metabolism, (ii) clonal architecture, depending on structural constraints (*structural blue-print sensu* Huber *et al*. 1999) and modalities of connection elongation *vs.* branching, and (iii) modalities of resource translocation and storage. Our purpose was to focus on clonal growth forms described by clonal architecture, resource translocation and storage. Consequently, amongst these 16 input parameters, only those related to points (ii) and (iii) were tested. Values of the four parameters related to point (i) were fixed on the basis of previous results

(Mony *et al*. submitted). These parameters, here onwards referred to as *clonal traits*, are presented in Table 1.

Table 1 - Parameters related to the clonal growth, the defoliation pattern and the impact of defoliation on the clonal growth. See Appendix A for more details on the meaning of the parameters and the equations in which they are involved.

Name	Meaning	Values
Basic metabolism		
r_p	Rate of energy gain by a ramet growth unit (L1 and L2)	0.15
C_r	Cost of production of a ramet growth unit (L1)	1
C_c	Cost of production of a connection growth unit (L1)	0.5
p_{g0}	Threshold of the probability for the production of a new unit (L11)	0.4
Clonal architecture		
	Structural constraints	
n_i	Maximal number of buds of the parent ramet growth unit	2 – 4 – 6
n_b	Maximal number of buds of a ramet growth unit	1 – 2
d_0	Mean number of connection units for a spacer (L4)	1 – 2 – 4
d_1	Maximum number of connection units that can be added to d_0 (L4)	0 – 1 – 2
	Modalities of elongation vs. branching	
$p_{el/br(0)}$	Threshold value for elongation versus branching process (L5)	0.2 – 0.8
E_g	Dependence of elongation on the generation number (L6)	0.05 - 2
E_l	Dependence of elongation on the length of the branch (L6)	0.0125 – 0.1
B_g	Dependence of branching on the generation number (L7)	0.05 - 2
B_l	Dependence of branching on the length of the branch (L7)	0.0125 – 0.1
B_p	Dependence of the branching process along the branch on the length of the branch (L8)	0.01 – 0.1
Modalities of resource translocation and storage		
d_r	Number of growth units of an IPU (L3)	2 – 10 – 99
r_s	Proportion of energy stored in connection units of the IPU	0 – 0.2 – 0.5
Patterns of defoliation		
P	Percentage of defoliated cells	10 – 20 – 40
G	Mean number of cells clustered into a basic unit of defoliation	1 – 2 – 4 – 10 – 20
F	Number of time steps between two consecutive defoliation events	10 – 20 – 50
I	Proportion of biomass lost by a defoliated ramet unit (L9)	0.1 – 0.5 – 0.9

The defoliation pattern layer

The defoliation pattern layer is composed of defoliated and non-defoliated cells. Defoliation patterns rely on four defoliation parameters. The spatial arrangement of the defoliated cells, *i.e.* spatial heterogeneity of defoliation depends on (i) the *percentage* of defoliated cells (*P*) and (ii) the *grain*, *i.e.* mean number of cells clustered into a basic unit of defoliation (*G*). (iii) The *frequency* of defoliation is modeled as the number of time steps between two consecutive defoliation events (*F*). (iv) The *intensity* of defoliation corresponds to the proportion of biomass lost by a defoliated ramet unit (*I*). The timing of the first defoliation event depends on the parameter frequency (*F*). A defoliation event lasts for one time step. Defoliated cells are positioned randomly in the lattice, with respect to the percentage (*P*) and grain (*G*). This spatial position varies randomly between two consecutive defoliation events.

Link between genet and defoliation pattern layers

The parameter intensity (*I*) determines the interaction between the genet layer and the defoliation pattern layer. Defoliation affects only ramet growth units, which loose a quantity of biomass proportional to their biomass at the time of the defoliation, according to the parameter intensity (*I*). As they virtually develop below-ground or close to the ground surface, connection growth units cannot be defoliated. Consequently, if a defoliated cell is superimposed either to a connection growth unit or to an empty cell nothing occurs. Moreover, we did not model the possibility of connection breakage. Consequently, the proportion of energy stored in these connection growth units is saved from defoliation. Detailed processes and equations are provided in Appendix A.

Simulations

Each simulation corresponded to a singular combination of clonal traits. One simulation lasted for 100 time steps. Although no unit of time was explicitly expressed, one time step corresponded to about one day of growth. In order to determine the number of runs for one simulation, convergence of results was tested by a Monte-Carlo method. In that purpose, a simulation was repeated a number of times and the point of convergence was looked for. Results converged from 100 runs onward. Consequently, 100 runs of each simulation were done and the final result of a simulation corresponded to the average results of the 100 runs. Each simulation was initiated by the placement of a ramet growth unit in the center of the hexagonal lattice of the genet layer. Within each time step, genet growth was processed following the updating process detailed in appendix A. If a defoliation event occurred at a

given time step, it was processed first. The creation of a ramet or a connection growth unit at the end of a time step depended on both the laws and parameters related to clonal growth and to the defoliation pattern and the history of the genet. During genet growth, an empty cell can become either a ramet growth unit or a connection growth unit. Once the status of a growth unit was defined, it was fixed for the whole duration of the simulation. Ramet growth units could be created only in empty cells, while connection growth units could be created either in empty cells or cells already occupied by a ramet or another connection growth unit.

We selected 135 *defoliation patterns* corresponding to the combinations of several values of the defoliation parameters (3 P × 5 G × 3 F × 3 I, see Table 1). In addition, two control homogeneous patterns (C) were simulated: no defoliated cells (*C0*) and 100 % of defoliated cells (*C100*). For both patterns, the frequency and the intensity of defoliation are the only parameters to vary, the other parameters being fixed. For each defoliation pattern, there were 31,104 possible combinations of clonal traits ($3^5 \times 2^7$, see Table 1). This corresponded to about 4.2×10^6 simulations (*i.e.* 4.2×10^8 runs) to browse the whole space of clonal traits, which was expected to last for more than eight months. To reduce the duration of the simulation procedure, a Monte-Carlo method was applied: for all of the 135 defoliation patterns and the two controls, the simulations were carried out for only 10 % of combinations randomly chosen among all possible combinations of clonal traits (about 3,200 combinations randomly chosen). Consequently, the simulations ran about 4.3×10^7 times (137 patterns × 3200 combinations × 100 runs), which lasted for about one week.

Three variables related to genet performance were monitored at the end of each run and their average value was calculated for each simulation (*i.e.* 100 runs):

(i) The final biomass of the genet (***Biom***) was calculated as the sum of the biomass of each ramet and connection growth unit.

(ii) The final number of ramets (***Nram***) corresponded to the final number of ramet growth units. It informed on the efficiency of clonal multiplication.

(iii) The length of the longest connection (***Lmax***) corresponded to the number of connection growth units constituting the longest connection. It was used to estimate the lateral expansion of the genet.

For each of the 137 defoliation patterns, the simulations resulted into a matrix *MA* containing almost 3,200 rows, each row corresponding to one simulation (*i.e.* one combination of clonal traits) and 15 columns, corresponding to the 12 clonal traits and the three indicators of genet performance. From there onwards, the two control patterns were analyzed separately from the 135 other patterns.

Data analysis

Impact of the defoliation parameters on genet performance

From each of the 135 matrices, we extracted the average and the maximal values of the three indicators of genet performance. We created a matrix with 135 rows corresponding to the defoliation patterns, and 10 columns corresponding to the four defoliation parameters and the average and maximal values of the three indicators of performance. We tested the relative importance of each defoliation parameter on genet performance by GLM multiple regressions. For each linear multiple regression, defoliation parameters were introduced as explanatory variables. The dependent variables were the average and the maximal values of the three indicators of genet performance. Control patterns (*C0* and *C100*) could not be included in the regressions as they represent singular combinations of defoliation parameters.

Relationship between defoliation parameters and clonal traits

We grouped the 135 matrices *MA* into a single matrix *MB* (432,000 rows × 15 columns). We then divided the matrix *MB* according to the values of each defoliation parameter. For instance, regarding the percentage (*P*), the division of the matrix resulted into three subsidiary matrices *P10*, *P20* and *P40*, corresponding respectively to the three possible values of the percentage. We made the same manipulation for the three other defoliation parameters. Thus, we obtained 14 matrices (*P10*, *P20*, *P40*, *G1*, *G2*, *G4*, *G10*, *G20*, *F50*, *F20*, *F10*, *I01*, *I05* and *I09*).

In each matrix, we identified the simulations that had performed the best. In that purpose, we isolated from each matrix the simulations that had reached between 90 % and 100 % of the maximal value of either biomass, number of ramets or maximal length (respectively *Top10-Biom*, *Top10-Nram* and *Top10-Lmax*). We also isolated the Top10 simulations from the two control matrices, *C0* (no defoliation) and *C100* (homogeneous defoliation). In order to characterize the corresponding clonal growth forms, we analyzed the profile of distribution of clonal trait values that had enabled to reach the Top10 % of performance. To that purpose, we followed the method described by Mony *et al*. (submitted). Amongst the Top10 simulations and for each clonal trait, we counted the number of simulations corresponding to a given value. On this basis, we distinguished three kinds of clonal traits: (i) traits for which at least 90 % of the Top10 simulations were distributed in only one value of the trait (*unique-value traits*), (ii) traits for which the Top10 simulations were distributed a complex manner in several values of the trait (*complex-value traits*) and

(iii) traits for which the Top10 simulations were equi-distributed amongst all trait values (*equi-distributed traits*). We used the significance of Chi² tests to distinguish between complex-value and equi-distributed traits: $P < 0.05$ indicated complex distribution, $P > 0.05$ indicated equi-distribution. We applied this method for each defoliation parameters and for the three indicators of genet performance.

In order to investigate the impact of defoliation on these profiles of distribution, we grouped clonal traits into three categories. (i) Traits that have the same profile regardless the defoliation parameter were considered as *not involved* (*NI*) in the response to defoliation. Traits involved in the response to defoliation were classified into two groups. (ii) *Parameter-independent traits* (*PI*) were involved in the response to heterogeneous defoliation *vs.* homogeneous conditions. They showed contrasted profiles of distribution between control homogeneous patterns (no defoliation *C0* and homogeneous defoliation *C100*) on the one hand and heterogeneous patterns, regardless the parameter *P*, *G*, *F* and *I*, on the other hand. (iii) *Parameter-dependent traits* (*PD*) were involved in the response to defoliation depending on the parameter value. Their distribution depended on the values of defoliation parameter *P*, *G*, *F* and *I*.

All programming was done in C. GLM multiple regressions were carried out on R software (R Development Core Team, 2007, http://www.R-project.org).

Results

Impact of the defoliation parameters on genet performance

The percentage of defoliated cells (*P*) influenced genet performance the most strongly (Table 2). Both average and maximal values of genet biomass (*Biom*) declined as the percentage of defoliated cells increased (Fig. 1a, b). In contrast, for both the final number of ramets (*Nram*) and the length of the longest connection (*Lmax*), the average values depended on the percentage of defoliated cells, while the maximal values did not. However, the adjusted R^2 obtained for the average values of *Nram* and *Lmax* were low (respectively 0.06 and 0.04; Table 2), suggesting that, despite significant *P*-values, the dispersion was great and the regressions little reliable (Fig. 1c, d). Defoliation grain (*G*), frequency (*F*) and intensity (*I*) had no effect on *Nram* nor *Lmax* (Table 2). Defoliation grain (*G*) affected however the average value of *Biom*, and defoliation intensity (*I*) impacted the maximal value of this indicator (Table 2). However, the values of R^2 were very low, indicating little reliable relationships (Fig. 1e, f).

Table 2 – Results of the multiple regressions of the average and maximal value of the indicators of genet performance against the defoliation parameters. ns P > 0.05, * P < 0.05, ** P < 0.01, *** P < 0.001. *Biom*: genet biomass, *Nram*: number of ramets, *Lmax*: length of the longest connection.

	Biom		*Nram*		*Lmax*	
	Mean	Max	Mean	Max	Mean	Max
Percentage (*P*)	***	***	***	ns	**	ns
Grain (*G*)	*	ns	ns	ns	ns	ns
Frequency (*F*)	ns	ns	ns	ns	ns	ns
Intensity (*I*)	ns	*	ns	ns	ns	ns
Adjusted R^2	0.76	0.33	0.06	0.02	0.04	0.00

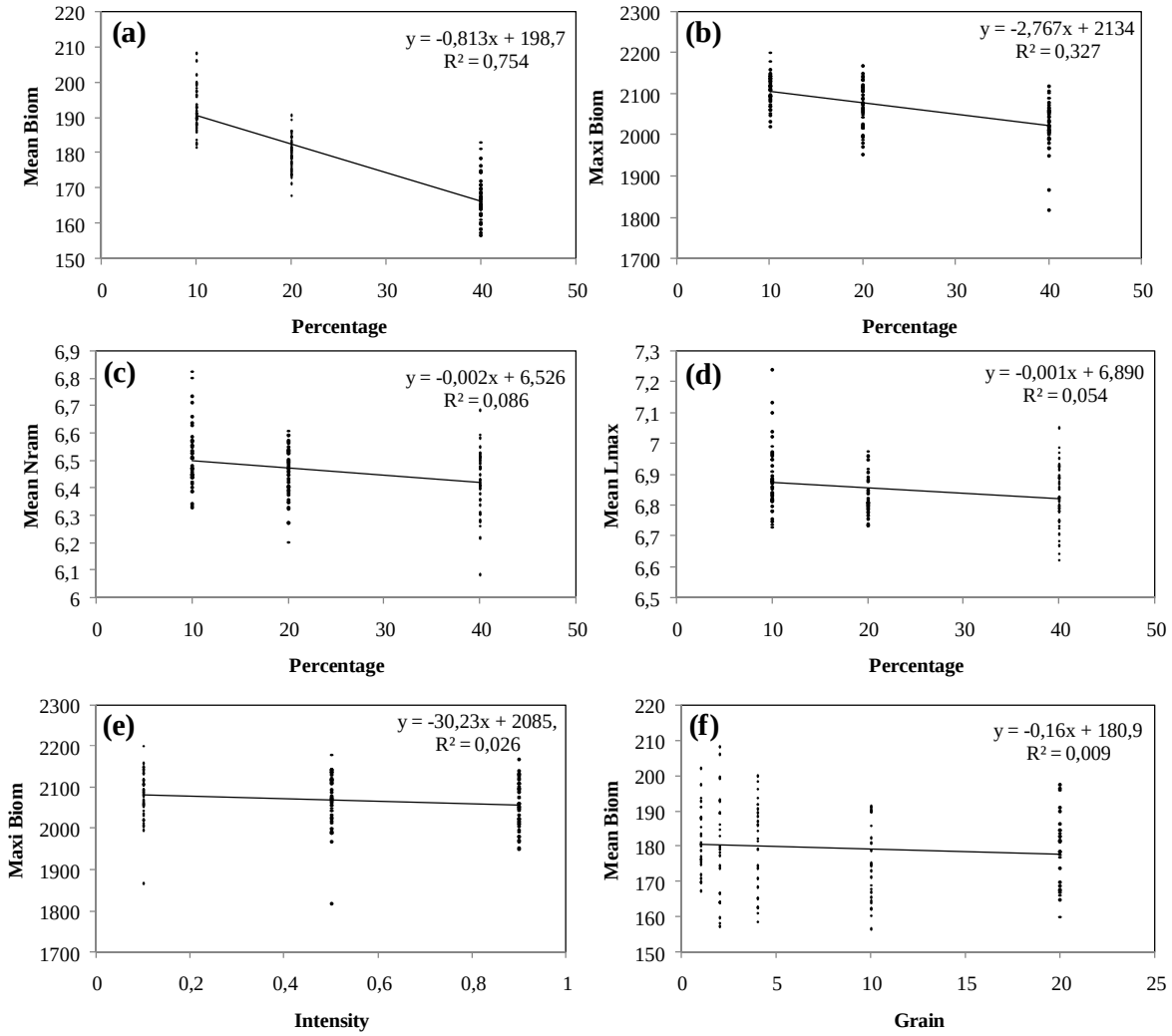

Fig. 1 – Univariate linear regressions between the defoliation parameters and the indicators of genet performance. Only the relations emerging significant from the multiple regression GLMs are represented.

While the average and maximal values of *Biom* tended to be greater in the absence of defoliation (*C0*) than under homogeneous defoliation (*C100*), the differences were much weaker for *Nram* and *Lmax* (Table 3).

Table 3 – Average and maximal values of the indicators of genet performance for the two control patterns. *C0*: no defoliation, *C100*: homogeneous defoliation.

	Biom		*Nram*		*Lmax*	
	Mean	Max	Mean	Max	Mean	Max
C0	200.93	2204.43	6.43	31.43	6.80	23.29
C100	114.00	2020.95	6.40	31.35	6.77	22.87

Relationship between defoliation parameters and clonal traits

For all of the three indicators of genet performance, only a few simulations reached the Top10 % of performance, regardless the defoliation parameter. Amongst all simulations, the percentage of simulations reaching the Top10 % of performance was comprised between 0.03 and 0.35 % for the indicator *Biom*, between 0.59 and 0.75 % for *Nram* and between 1.32 and 1.70 % for *Lmax* (Fig. 2). For the indicator *Biom*, the percentage of simulations reaching the Top10 % of performance was the most important for the control without defoliation (*C0*), intermediate for heterogeneous defoliation, regardless the values of the defoliation parameters, and the smallest for the control with homogeneous defoliation (*C100*; Fig. 2).

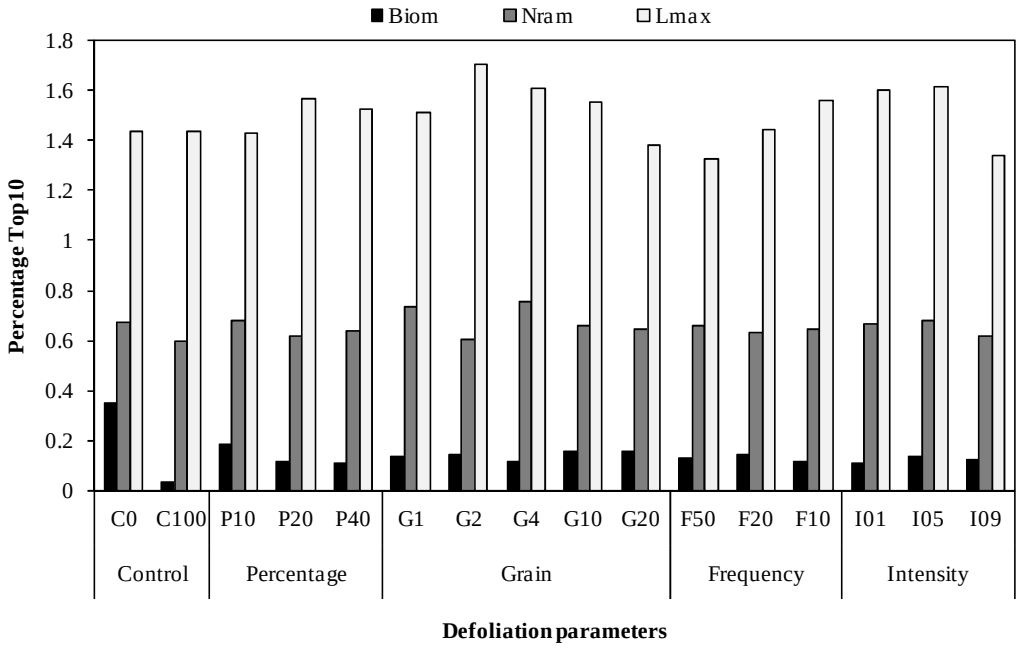

Fig. 2 – Percentage of simulations (combinations of clonal traits) reaching the Top10 for the three indicators of genet performance, according to the defoliation parameters. *Biom*: genet biomass, *Nram*: number of ramets, *Lmax*: length of the longest connection.

Regarding the indicator *Biom*, seven clonal traits were not involved in the response to defoliation (*NI*), three traits were involved in the response to heterogeneous defoliation *vs.* no and homogeneous defoliation, but independently from the parameter values (parameter-independent traits, *PI*) and two traits were involved in the response to defoliation, depending the parameter values (parameter-dependent traits, *PD*; Table 4).

1- *NI* traits. Amongst traits that were not involved in the response to defoliation, four traits were equi-distributed, indicating that their value had no importance for the maximization of biomass. These traits were related to the laws governing the branching processes (B_g, B_l and B_p) and the allocation to reserves (r_s). One trait was complex-value (E_g) and two traits were unique-value (E_l and $p_{el/br(0)}$). For these three traits, which are involved in the elongation processes, low values were the most represented.

2- *PI* traits. Heterogeneous defoliation, whatever the parameter considered, significantly impacted inter-ramet distances: d_0 shifted from the unique value of 1 to either a complex value or a unique value of 4; d_1 shifted from the unique value of 1 to a complex value. Consequently, while no defoliation or homogeneous defoliation favored a distance inter-ramet of 1 ± 1, defoliation favored longest inter-ramet distances with low variability. Moreover, while no defoliation favored high numbers of buds at the parent ramet (complex value of n_i), this parameter shifted to equi-distribution when defoliation occurred.

3- *PD* traits. The number of buds of ramet growth units (n_b) and the distance of resource sharing (d_r) responded in a complex manner to defoliation and were either complex value or equi-distributed, depending on the defoliation parameter considered.

Similar results are found when the indicator of genet performance is *NbRam* (Table 5).

1- *NI* traits. Independently from the defoliation parameter, B_g, B_l and r_s were equi-distributed, E_g and n_b were complex-value and E_l and $p_{el/br(0)}$ were unique-value. Low values of E_g, E_l and $p_{el/br(0)}$, which promote the elongation processes, and high values of n_b (*i.e.* great number of potential branch connections) were favored.

2- *PI* traits. Three traits involved in the response to heterogeneous defoliation were parameter-independent (*PI*). Control patterns were characterized by small and little variable inter-ramet distances (d_0 and d_1) and small distances of resource sharing (d_r). By contrast, heterogeneous defoliation promoted long and either invariable or little variable inter-ramet distances (d_0 and d_1), indifferently to the distance of resource sharing (d_r was equi-distributed).

Table 4 – Profiles of distribution of clonal traits for simulations maximizing genet biomass (*Top10-Biom*). Equi-distributed traits are symbolized by Ø. Complex-value traits are symbolized by arrows, the direction designing the most represented value (↗ high; → intermediate; ↘ low values). Unique-value traits are represented by their value. NI: traits not involved in the response to defoliation; PI: parameter-independent traits; PD: parameter-dependant traits.

	Homogeneous controls		Heterogeneous defoliation														
			Percentage			Grain					Frequency			Intensity			
	C0	*C100*	*P10*	*P20*	*P40*	*G1*	*G2*	*G4*	*G10*	*G20*	*F50*	*F20*	*F10*	*I01*	*I05*	*I09*	
Modalities of resource translocation and storage																	
d_r	↘↗	Ø	Ø	↗	Ø	Ø	↗	Ø	Ø	Ø	Ø	Ø	Ø	↘↗	Ø	Ø	PD
r_s	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	→	Ø	Ø	NI
Structural constraints																	
n_i	↗	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	PI
n_b	Ø	Ø	Ø	↗	Ø	↗	Ø	Ø	Ø	Ø	Ø	Ø	↗	↗	Ø	Ø	PD
d_0	1	1	↗	↗	4	4	→	→	4	↗	↗	↗	↗	↗	↗	↗	PI
d_1	1	1	↘	↘	→	→	↘	↘	→	→	↘	↘	↘	↘	↘	↘	PI
Modalities of elongation vs. branching																	
$p_{el/br(0)}$	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	NI
E_g	↘	Ø	↘	↘	↘	↘	↘	↘	↘	↘	↘	↘	↘	↘	↘	↘	NI
E_l	0.0125	0.0125	0.0125	0.0125	0.0125	0.0125	↘	↘	0.0125	0.0125	0.0125	0.0125	0.0125	0.0125	0.0125	0.0125	NI
B_g	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	NI
B_l	Ø	Ø	↗	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	↗	Ø	Ø	NI
B_p	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	↗	Ø	Ø	Ø	Ø	Ø	Ø	Ø	NI

Table 5 – Profiles of distribution of clonal traits for simulations maximizing the number of ramets (*Top10-Nram*). See Table 4 for the meaning of symbols and abbreviations.

	Homogeneous controls		Heterogeneous defoliation													
			Percentage			Grain					Frequency			Intensity			
	C0	*C100*	*P10*	*P20*	*P40*	*G1*	*G2*	*G4*	*G10*	*G20*	*F50*	*F20*	*F10*	*I01*	*I05*	*I09*	
Modalities of resource translocation and storage																	
d_r	↘	↘	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	PI
r_s	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	NI
Structural constraints																	
n_i	↗	↗	Ø	Ø	Ø	↘	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	→	PD
n_b	↗	↗	↗	↗	↗	↗	↗	↗	↗	↗	↗	↗	↗	↗	↗	↗	NI
d_0	1	1	↗	↗	4	4	↗	↗	4	↗	↗	↗	↗	↗	↗	↗	PI
d_1	1	1	→	→	→	→	→	↘	→	→	→	→	→	→	→	→	PI
Modalities of elongation vs. branching																	
$p_{el/br(0)}$	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	NI
E_g	↘	↘	↘	↘	↘	↘	↘	↘	↘	↘	↘	↘	↘	↘	↘	↘	NI
E_l	0.0125	0.0125	0.0125	0.0125	0.0125	0.0125	0.0125	0.0125	0.0125	0.0125	0.0125	0.0125	0.0125	0.0125	0.0125	0.0125	NI
B_g	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	NI
B_l	Ø	Ø	↘	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	NI
B_p	Ø	Ø	Ø	Ø	↘	Ø	Ø	Ø	↘	Ø	↘	Ø	Ø	↘	Ø	Ø	PD

Table 6 – Profiles of distribution of clonal traits for simulations maximizing the length of the longest rhizome (*Top10-Lmax*). See Table 4 for the meaning of symbols and abbreviations.

	Homogeneous controls		Heterogeneous defoliation													
			Percentage			Grain					Frequency			Intensity			
	C0	*C100*	*P10*	*P20*	*P40*	*G1*	*G2*	*G4*	*G10*	*G20*	*F50*	*F20*	*F10*	*I01*	*I05*	*I09*	
Modalities of resource translocation and storage																	
d_r	↘	↘	Ø	Ø	Ø	Ø	Ø	↗	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	PI
r_s	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	NI
Structural constraints																	
n_i	↘	↘	→	Ø	Ø	Ø	→	Ø	Ø	Ø	Ø	Ø	Ø	→	Ø	Ø	PD
n_b	↘	↘	↘	↘	↘	↘	↘	↘	↘	↘	↘	↘	↘	↘	↘	↘	NI
d_0	↗	Ø	Ø	→	→	→	Ø	↘	→	Ø	Ø	→	→	→	→	Ø	PD
d_1	Ø	↘	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	NI
Modalities of elongation vs. branching																	
$p_{el/br(0)}$	↘	↘	Ø	↗	↗	↗	Ø	Ø	↗	↗	↗	↗	↗	↗	↗	↗	PD
E_g	2	↗	2	2	↗	↗	↗	↗	↗	2	2	2	↗	↗	↗	2	NI
E_l	0.0125	0.0125	0.0125	0.0125	0.0125	0.0125	0.0125	0.0125	0.0125	0.0125	0.0125	0.0125	0.0125	0.0125	0.0125	0.0125	NI
B_g	Ø	↘	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	NI
B_l	↘	↘	↘	↘	↘	↘	Ø	Ø	↘	↘	↘	Ø	↘	↘	Ø	Ø	PD
B_p	↗	↗	Ø	Ø	Ø	↗	↗	Ø	↗	Ø	Ø	Ø	Ø	Ø	Ø	Ø	PD

3- *PD* traits. Two traits responded in a complex manner to defoliation (*PD*). While great numbers of potential primary connections (n_i) were promoted in the absence of defoliation, this trait became either complex-value or equi-distributed when defoliation occurred. Depending on the defoliation parameter, B_p was either equi-distributed or complex-value.

Results obtained when the indicator of performance considered was *Lmax* differed from the results described above (Table 6). An important set of clonal traits was complexly involved in the response to defoliation, their profile of distribution depending on the defoliation parameter.

1- *NI* traits. Regardless the defoliation parameter, B_g and r_s remained equi-distributed and thus not involved in the maximization of *Lmax*. By contrast, *Lmax* was maximized by a small number of potential branch connections (n_b), high values of E_g and low values of E_l: the elongation processes was disfavored for connections of high orders, while it was independent of connection length.

2- *PI* traits. Among traits involved in the response to heterogeneous defoliation, d_r emerged as the only parameter-independent. The absence of defoliation favored integration on small distance while the distance of integration had no importance in the maximization of *Lmax* when defoliation occurred.

3- *PD* traits. Several traits including traits related to structural constraints (n_i, d_0 and d_1) and to patterns of elongation *vs.* branching (B_l, B_p and $p_{el/br(0)}$) had a complex behavior in response to defoliation. However, d_1 and B_p tended to be qui-distributed whatever the defoliation parameter, suggesting their rather insignificant implication in the maximization of *Lmax*.

Discussion

Impact of defoliation on genet performance

We expected that, amongst all possible clonal growth forms (*i.e.* combinations of clonal traits), at least one would enable to maximize genet performance. Our results demonstrate that the impact of defoliation depended on the indicator of genet performance, as well as on the defoliation parameter considered.

Genet biomass was the indicator of performance the most affected by defoliation. Indeed, defoliation has often been shown to decrease the biomass of individual plants compared to plants of the same species grown in the absence of defoliation (see for instance Price & Hutchings 1992b, Hicks & Turkington 2000, Ferraro & Oesterheld 2002). By contrast, both maximal and average values of the number of ramets and length of the maximal rhizome remained unchanged regardless defoliation parameters. These results contrast with empirical observations of limited clonal expansion following defoliation (Moen *et al*. 1999, Piqueras *et al*. 1999, Wang *et al*. 2004, Henry *et al*. 2007). In our model, defoliation was simulated as the removal of above-ground biomass, after which the ramet accumulates resources again. Such impact of defoliation is generally applied in models at diverse scales, from the organ (*e.g.* Tomlinson *et al*. 2007) to the plant community (*e.g.* Mouissie *et al*. 2008). However, our model considered two hierarchical levels: the growth unit (ramet and connection) and the genet. Resources accumulated by the ramet after defoliation are immediately shared between growth units, depending on the distance of resource sharing, and available for genet growth (*i.e.* the production of new growth units). By contrast, compensatory growth at the ramet scale consists in the reformation of damaged tissues (Richards 1993). Such diversion of resources may occur at the expense of genet growth and has been suggested to decrease current elongation of existing connections or production of new ones (Stoll *et al*. 1998, Meyer and Schmid 1999). In our model, losses of resources may have been weak enough to allow the development of the connection network and the production of ramets, regardless the values of defoliation parameters. The simulation of a delay in growth or a threshold of ramet biomass, below which it cannot share resources for genet growth, may have limited genet growth (*i.e.* production of ramets and/or elongation of connections).

Biomass was particularly driven by the percentage of defoliated cells, while it was unaffected by the grain, frequency and intensity of defoliation. In simulation studies, the quality of habitats is often described as the percentage of resource-rich patches (see for instance Oborny *et al*. 2001, Oborny & Kun 2002, Kun & Oborny 2003). Similarly, the percentage of defoliated cells determines at first the severity of defoliation at the genet scale, as it is closely linked with the percentage of ramets that may be defoliated. By contrast, frequent or intense defoliation might cause an important loss of resources at the ramet scale, which could be compensated for by resource translocation amongst ramets. Contrasting with our results, responses of clonal plants to environmental heterogeneity have been suggested to depend on its grain (Wijsinghe & Hutching 1997, Alpert & Simms 2002). Plastic adjustments

could have promoted the escape of ramets from defoliated patches and enhanced genet performance, providing that the grain was perceptible at the genet scale (Stuefer 1996, Wijesinghe & Hutchings 1997). The impact of the grain on genet biomass was certainly underestimated in our model as no phenotypic plasticity has been simulated.

Impact of defoliation parameters on clonal traits

The number of simulations (combinations of clonal traits) that enabled to maximize genet performance, *i.e.* that reached the Top10 % of performance, was less than 2 % of the whole set of simulations. It depended on the indicator of genet performance: it was the greatest for the length of the longest connection, intermediate for the number of ramets and the lowest for the biomass. The very small number of simulations that succeeded in maximizing biomass suggests that this indicator depended strongly on few combinations of clonal traits. However, this result could be biased by the range of values for biomass (from 0 to more than 2200), which was hugely greater than the range of values for the number of ramets or the length of the longest connection (inferior to 35 and to 25, respectively). Biomass was the only indicator for which the number of simulations reaching the Top10 % of performance was sensitive to defoliation, in particular to its spatial heterogeneity. The combinations of clonal traits maximizing the biomass under homogeneous defoliation were rare, while heterogeneous defoliation and, to a larger extent, no defoliation allowed more diverse clonal growth forms to reach high values of biomass. Thus, homogeneous defoliation, regardless its frequency and its intensity, emerged as a strong filter on clonal traits.

Architectural traits

The growth forms that maximized the number of ramets were closely similar to those maximizing biomass, regardless the defoliation parameter. Traits related to the structural blue-print were of great importance in the response to defoliation. Surprisingly, although homogeneous defoliation acted as a stricter filter than no defoliation (see above), both control patterns selected for convergent traits, which contrasted with those under heterogeneous defoliation. However, none trait related to the modalities of connection elongation *vs.* branching was involved in the response to defoliation. Contrary to our hypothesis that elongation should be promoted over branching under heterogeneous defoliation, this occurred regardless the characteristics and even the presence of defoliation. Branching pattern could vary without altering the genet biomass, possibly because of the rare occurrence of branching. The field parameterized simulation model carried out by Wildová *et al.* (2007) on six clonal

sedges led to a similar conclusion, the probability of non-terminal branching being uncorrelated with total biomass for five species.

Homogeneous conditions favored great numbers of potential primary connections with small spacers. The resulting clonal growth forms promote at the same time centrifugal spread through the elongation of numerous primary connections, and ramet density through small spacer lengths (Herben & Suzuki 2002). Such clonal architectures are expected to maximize spatial occupation, while limiting the investment in the connection network. In particular, the resulting population of ramets seems dense enough to prevent the intrusion of competitors (Smith & Palmer 1976, Callaghan *et al*. 1990). By contrast, heterogeneous defoliation, regardless its grain, frequency or intensity, promoted long spacers. In addition to an important investment in the elongation of connections, long spacers enhance the exploration of space and the probability to encounter new conditions (Ikegami *et al*. 2007). In heterogeneous defoliation, this strategy may allow dispersed ramets to spread the risk of being damaged (Piqueras 1999). Dispersion may imply costs to the genet, notably related to the production and maintenance of connections (de Kroon & Schieving 1990, van Groenendael *et al*. 1996). It is all the more true in our model, where the creation of connection growth units consumes resources, whereas they are not able to acquire resources for further growth. Connections are only involved in lateral expansion and resource storage. When the environment is heterogeneous, it is not worth investing in dispersion and the individual may benefit more from exploiting the place it occupies.

Physiological traits: resource storage and translocation

Several physiological studies of recovery after defoliation have demonstrated that stored resources are implied readily after defoliation to enable a rapid refoliation and the restoration of efficient photosynthesis (Richards 1993, de Visser *et al.* 1997, Morvan-Bertrand *et al.* 1999a, b). These benefits are expected to outweigh the decrease in current growth rate often associated with the diversion of resources to storage (Chapin *et al*. 1990, Kobe 1997, van der Meidjen *et al.* 2000, de Jong & van der Meijden 2000). Consequently, storage should be advantageous in response to frequent or intensive defoliation. Contrary to this expectation, the proportion of resources allocated to storage had no importance in genet success, regardless the indicator of performance as well as the defoliation parameter. In the present study, storage was modeled as a simple process of resource translocation from ramet to connection growth units. As connection growth units could not be defoliated, storage enabled the genet to save some amount of resource from defoliation. Moreover, resources stored in the connections

were readily available for current growth, either a defoliation event had occurred or not. Consequently, the cost allocated to storage was low. A first explanation of the unimportance of storage in response to defoliation may rely on the fact that we did not model compensatory growth *sensu stricto*, as we did not pay attention to the regrowth of single ramets. Losses of biomass might have been light enough to allow the development of the genet without support from reserves. Moreover, the advantage of reserve making likely relies on its involvement in rapid regrowth, enhancing the competitive ability of the plant individual over its neighbors immediately after defoliation. This benefit has probably been alleviated by the conditions of simulations where the genet was modeled alone, without competing neighbors.

The distance of resource sharing, as well as the potential number of branch connections, were complexly involved in the response to defoliation, depending on the parameter values. Extensive resource sharing was promoted by intermediate percentage of defoliation and fine grain, as it could enhance the support of defoliated ramets by undamaged ones (Jónsdóttir & Callaghan 1989). Such risk sharing strategy (*sensu* Oborny *et al*. 2000) may enable the genet to buffer damage. Our results indicated that, otherwise, the modalities of physiological integration did not matter in the response to heterogeneous defoliation. Extensive physiological integration has been suggested to be costly to the plant, notably because the maintenance of functionally active vessels requires energy (Caraco & Kelly 1990, Kelly 1995). In our model, although the production of connection growth units consumes resources, their maintenance was not implemented. In situations where physiological integration is not necessary, a cost of maintenance might have disfavored extensive resource sharing and led to different results

Importance of the indicator of performance investigated

Architectural traits were more involved in the optimization of the number of ramets than of biomass. In particular, the number of ramets was maximized by a great number of potential branch rhizomes. Yet, clonal growth forms maximizing genet biomass and ramet number were similar. Consequently, genet biomass and number of ramets appeared as reliable indicators of genet performance. Contrasting with this convergence, optimal clonal growth forms were different when the length of the longest connection was considered. At first glance, this is in contradiction with the positive trade-off between ramet number and rhizome length demonstrated by Wildová *et al*. (2007a). However, this difference may in part be due to our choice to estimate performance through the length of only one (the longest) connection rather than total connection length. The number of long connections within a genet may vary

without altering the length of the longest one, but genet biomass and number of ramets, explaining the absence of link between clonal growth forms maximizing the former *vs.* the latter indicators of performance. It is important to note that there seems to be little consistency between the clonal growth forms maximizing lateral propagation and the characteristics of defoliation. Although the length of the longest connection provides information on the capacity of clonal spread in one direction, it does not describe the efficient propagation in the whole horizontal plan. This estimation could be improved by other indicators such as total length of connections or final area covered by the genet.

Perspectives and improvements of the model

The present study provides insights in the implications of clonal growth forms in response to defoliation. Experimental studies assessing clonal responses to defoliation often focus on one or two of these characteristics such as defoliation frequency (Archer & Delting 1984), intensity (Hicks & Turkington 2000, Wang *et al*. 2004), or spatial pattern (Jónsdóttir & Callaghan 1989, Hartnett 1989, Price & Hutchings 1992b), but they do not allow to test for more complex combinations. Moreover, these experimental tests, dealing with one or several species, are conditioned by linkages between traits in an organism (Reich *et al*. 1999, Wildová *et al*. 2007a). The modeling approach has enabled to test for combinations of several parameters and, consequently, to disentangle the relevance of clonal traits in response to precise defoliation characteristics. However, our results highlighted some limits in the model, which should be further considered and improved.

Optimal growth forms were consistent when genet performance was estimated through genet biomass and number of ramets, but differed when the length of the longest connection was used as the estimator of performance. Estimation of individual fitness is a complex task, which is made all the more difficult in clonal plants because of their hierarchical organization (Tuomi & Vuorisalo 1989, Wikberg 1995). The choice of several indicators, related to the genet and the ramet population, emerges as one way to solve these difficulties and to obtain a sharp evaluation of genet performance. However our results proved that this choice must be considered with caution. Moreover, Wildová *et al*. (2007a) showed the existence of trade-offs between components of genet performance, demonstrating that a genet had not the capability to optimize all of the indicators. Conclusively, the determination of a synthetic indicator of performance could be more reliable, whereas its relevance might depend on environmental conditions (Wildová *et al*. 2007a).

Contrary to our expectations, clonal traits related to modalities of resource translocation and storage were little or even not involved in the response to defoliation. Although it is tempting to conclude that architectural traits, notably trait related to structural blue-print, were the best predictors of the response of clonal plants to defoliation characteristics, these observations might be partly driven by the model itself. In particular the model focused on the response of a single genet to contrasted characteristics of defoliation, while plant response to defoliation in natural conditions likely depend on inter-specific interactions and the responses of neighbor plant individuals to similar defoliation.

Two further steps are considered to improve the present study:

(i) The creation of a synthetic indicator of genet performance could facilitate the interpretation of results and the description of clonal growth forms selected by environmental factors.

(ii) The modeling of a prairie, *i.e.* of several genets interacting with each other would enable to test the relevance of combinations of clonal traits in response to the direct (loss of above-ground biomass) and indirect effects of defoliation (canopy opening and changes in inter-specific interactions).

Literature cited

Adler PB, Raff DA & Laurenroth WK (2001) The effect of grazing on the spatial heterogeneity of vegetation. *Oecologia*, 128: 465-479.

Alpert P (1991) Nitrogen sharing among ramets increases clonal growth in *Fragaria Chiloensis*. *Ecology*, 72: 69-80.

Alpert P & Simms EL (2002) The relative advantages of plasticity and fixity in different environments: when is it good for a plant to adjust? *Evolutionary Ecology*, 16: 285-297.

Archer S & Delting JK (1984) The effect of defoliation and competition on regrowth of tillers of two North American mixed-grass prairie graminoids. *Oikos*, 43: 351-357.

Asaeda T, Rajapakse L, Manatunge J & Sahara N (2006) The effect of summer harvesting of *Phragmites australis* on growrth characteristics and rhizome resource storage. *Hydrobiologia*, 553: 327-335.

Bell AD (1984) Dynamic morphology: a contribution to plant population ecology. In: Dirzo R & Sarukhán J (eds) *Perspectives in Plant Population Ecology*. Sinauer, Sunderland, Massachusetts, pp 48-65.

Bullock JM, Franklin J, Stevenson MJ, Silvertown J, Coulson SJ, Gregory SJ & Softs R (2001) A plant trait analysis of responses to grazing in a long-term experiment. *Journal of Applied Ecology*, 38: 253-267.

Callaghan TV, Svensson BM, Bowmann H, Lindley DK & Carlsson BA (1990) Models of clonal plant growth based on population dynamics and architecture. *Oikos*, 75: 257-269.

Caraco T & Kelly CK (1990) On the adaptive value of physiological integration in clonal plants. *Ecology*, 72: 81-93.

Chapin FS III, Schulze ED & Mooney HA (1990) The ecology and economics of storage in plants. *Annual Review of Ecology and Systematics*, 21: 423-447.

de Jong T & van der Meijden E (2000) On the correlation between allocation to defence and regrowth in plants. *Oikos*, 88: 503-508.

de Kroon H & Schieving F (1990) Resource partitioning in relation to clonal growth strategy. In: van Groenendael J & de Kroon H (eds) *Clonal growth in plants: regulation and function*. SPB Academic Publishing, The Hague, pp 113-130.

de Kroon H & Hutchings MJ (1995) Morphological plasticity in clonal plants: the foraging concept reconsidered. Journal of Ecology, 83: 143-152.

de Visser R, Vianden H & Schnyder H (1997) Kinetics and relative significance of remobilized and current C and N incorporation in leaf and root growth zones of *Lolium perenne* after defoliation: assessment by ^{13}C and ^{15}N steady-state labelling. *Plant, Cell and Environment*, 20: 37-46.

Diaz S, Cabido M & Casanoves F (1998) Plant functional traits and environmental filters at a regional scale. *Journal of Vegetation Science*, 9: 113-122.

Ferraro DO & Oesterheld M (2002) Effect of defoliation on grass growth. A quantitative review. *Oikos*, 98: 125-133.

García MB & Ehrlén J (2002) Reproductive effort and herbivory timing in a perennial herb: fitness components at the individual and population levels. *American Journal of Botany*, 89, 1295-1302.

Hartnett DC (1989) Density- and growth stage-dependent responses to defoliation in two rhizomatous grasses. *Oecologia*, 80: 414-420.

Henry GM, Burton MG & Yelverton FH (2007) Effect of mowing on lateral spread and rhizome growth of troublesome *Paspalum* species. *Weed Science*, 55: 486-490.

Herben T & Suzuki JI (2002) A simulation study of the effects of architectural constraints and resource translocation on population structure and competition in clonal plants. *Evolutionary Ecology*, 15: 403-423.

Hicks S & Turkington R (2000) Compensatory growth of three herbaceous perennial species: the effect of clipping and nutrient availability. *Canadian Journal of Botany*, 78: 759-767.

Huntly N (1991) Herbivores and the dynamic of communities and ecosystems. *Annual Review of Ecology and Systematics*, 22: 477-503.

Hutchings MJ (1999) Clonal plants as cooperative systems: benefits in heterogeneous environments. *Plant Species Biology*, 14: 1-10.

Ikegami M, Whigham DF & Werger MJA (2007) Responses of rhizome length and ramet production to resource availability in the clonal sedge *Scirpus olneyi* A. Gray. *Plant Ecology*, 189: 247-259.

Iwasa Y & Kubo T (1997) Optimal size of storage for recovery after unpredictable disturbances. *Evolutionary Ecology*, 11: 41-65.

Jónsdóttir IS & Callaghan TV (1989) Localized defoliation stress and the movement of ^{14}C-photoassimilates between tillers of *Carex bigelowii*. Oikos, 54: 211-219.

Kelly CK (1995) Thoughts on clonal integration: facing the evolutionary context. *Evolutionary Ecology*, 9: 575-585.

Kleijn D & van Groenendael J (1999) The exploitation of heterogeneity by a clonal plant in habitats with contrasting productivity levels. *Journal of Ecology*, 87: 873-884.

Kleijn D, Treier UA & Müller-Schärer H (2005) The importance of nitrogen and carbohydrate storage for plant growth of the alpine herb *Veratum album*. *New Phytologist*, 166: 565-575.

Klimeš L, Klimešovà J, Hendricks R & van Groenendael J (1997) Clonal plant architecture: a comparative analysis of form and function. In: de Kroon H & van Groenendael J (eds) *The ecology and evolution of clonal plants*. Backhuys Publishers, Leiden, pp 1-29.

Kobe RK (1997) Carbohydrate allocation to storage as a basis of interspecific variation in sapling survivorship and growth. *Oikos*, 226-233.

Kun A & Oborny B (2003) Survival and competition of clonal plant populations in spatially and temporally heterogeneous habitats. *Community Ecology*, 4: 1-20.

Lovett-Doust L (1981) Population dynamics and local specialization in a clonal perennial (*Ranunculus repens*). I. The dynamics of ramets in contrasting habitats. *Journal of Ecology*, 69:743-755.

Macek P, Lepš J (2003) The effect of environmental heterogeneity on clonal behaviour of *Prunella vulgaris* L. *Plant Ecology*, 168: 31-43

Meyer AH & Schmid B (1999) Experimental demography of rhizome populations of establishing clones of *Solidago altissima*. *Journal of Ecology*, 87: 42-54.

Moen J, Ingvarsson PK & Walton DWH (1999) Estimates of structural complexity in clonal plant morphology: comparisons of grazed and ungrazed *Acaena magellanica* rhizomes. *Canadian Journal of Botany*, 77: 869-876.

Mony C, Garbey M, Smaoui M & Benot ML (submitted) Optimal trait profiles for clonal growth in plant species: a modeling approach.

Morvan-Bertrand A, Boucaud J & Prud'homme MP (1999a) Influence of initial levels of carbohydrates, fructans, nitrogen, and soluble proteins on regrowth of *Lolium perenne* L. cv. Bravo following defoliation. *Journal of Experimental Botany*, 50: 1817-1825.

Morvan-Bertrand A, Pavis N, Boucaud J & Prud'homme MP (1999b) Partitioning of reserve and newly assimilated carbon in roots and leaf tissues of *Lolium perenne* during regrowth after defoliation : assessment by ^{13}C steady-state labelling and carbohydrate analysis. *Plant, Cell and Environment*, 22: 1097-1108.

Moussie AM, Apol MEF, Heil GW & van Diggelen R (2008) Creation and preservation of vegetation patterns by grazing. *Ecological Modelling*, 218: 60-72.

Oborny B, Kun A, Czárán T & Bokros S (2000) The effect of clonal integration on plant competition for mosaic habitat space. *Ecology*, 81: 3291-3304.

Oborny B, Czárán T & Kun A (2001) Exploration and exploitation of resources patches by clonal growth: a spatial model on the effect of transport between modules. *Ecological Modelling*, 141: 151-169.

Oborny B & Kun A (2002) Fragmentation of clones: how does it influence dispersal and competitive ability? *Evolutionary Ecology*, 15: 319-346.

Olff H & Ritchie ME (1998) Effects of herbivores on grassland plant diversity. *Trends in Ecology and Evolution*, 13: 261-265.

Piqueras J (1999) Herbivory and ramet performance in the clonal herb *Trientalis europara* L. *Journal of Ecology*, 87: 450-460.

Piqueras J, Klimeš L & Redbo-Torstensson P (1999) Modelling the morphological response to nutrient availability in the clonal plant Trientalis europaea L. Plant Ecology, 141: 117-127.

Price EAC & Hutchings MJ (1992b) Studies of growth in the clonal herb *Glechoma hederacea*. II. The effect of selective defoliation. *Journal of Ecology*, 80: 39-47.

Puijalon S, Bouma TJ, van Groenendael J & Bornette G (2008) Clonal plasticity of aquatic plant species submitted to mechanical stress: escape versus resistance strategy. *Annals of Botany*, 102: 989-996.

R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Reich PB, Ellsworth DS, Walters MB Vose JM, Gresham C, Volin JC & Bowman WD (1999) Generality of leaf trait relationships: a test across six biomes. *Ecology*, 80: 1955-1969.

Richards JH (1993) Physiology of plants recovering from defoliation. In: Baker MJ (ed) *Proceedings of the XVII International Grassland Congress*. SIR Publishing, Wellington, pp 85-94.

Slade AJ & Hutchings MJ (1987a) The effect of nutrient availability on foraging in the clonal herb *Glechoma hederacea*. *Journal of Ecology*, 75: 95-112.

Slade AJ & Hutchings MJ (1987b) The effects of light intensity on foraging in the clonal herb *Glechoma hederacea*. *Journal of Ecology*, 75: 639-650.

Smaoui M, Garbey M & Mony C (2008) Volunteer computing for ecology: the Virtual Prairie BOINC project. Paper presented in IEEE Asia-Pacific Services Computing Conference (IEEE APSCC 2008), Taiwan, pp 427-434.

Smith AP & Palmer JO (1976) Vegetative reproduction and close packing in a successional plant species. *Nature*, 261: 232-233.

Stoll P, Egli P & Schmid B (1998) Plant foraging and rhizome growth patterns of *Solidago altissima* in response to mowing and fertilizer application. *Journal of Ecology*, 86: 341-354.

Stuefer JF (1996) Potential and limitations of current concepts regarding the response of clonal plants to environmental heterogeneity. *Vegetatio*, 127: 55-70.

Sutherland WJ & Stillman RA (1988) The foraging tactics of plants. Oikos, 52: 239-244.

Suzuki JI & Stuefer J (1999) On the ecological and evolutionary significance of storage in clonal plants. *Plant Species Biology*, 14: 11-17.

Tomlinson KW, Dominy JG, Hearne JW & O'Connor TG (2007) A functional-structural model for growth of clonal bunchgrasses. *Ecological Modelling*, 202: 243-264.

Tuomi, J. & Vuorisalo, T. (1989) What are the units of selection in modular organisms? Oikos, 54, 227-233.

van der Meidjen E, de Boer NJ & van der Veen-van Wijk (2000) Pattern of storage and regrowth in ragwort. *Evolutionary Ecology*, 14: 439-455.

van Groenendael J & de Kroon H (1990) *Clonal growth in plants: regulation and function.* SBP Academic Publishing, The Hague.

van Groenendael JM, Klimeš L, Klimešovà J & Hendricks R (1996) Comparative ecology of clonal plants. *Philosophical Transactions of the Royal Society London*, 351: 1331-1339.

Wang Z, Li L, Han X & Dong M (2004) Do rhizome severing and shoot defoliation affect clonal growth of *Leymus chinensis* at ramet population level ? *Acta Oecologica*, 26: 255-260.

Wijesinghe DK & Hutchings MJ (1997) The effects of spatial scale of environmental heterogeneity on the growth of a clonal plant: an experimental study with *Glechoma hederacea*. *Journal of Ecology*, 85: 17-28

Wikberg S (1995) Fitness in clonal plants. *Oikos*, 72: 293-297.

Wildová R, Gough L, Herben T, Hershock C & Goldberg DE (2007a) Architectural and growth traits differ in effects on performance of clonal plants: an analysis using a field-parameterized simulation model. *Oikos*, 116: 836-852.

Appendix A – Model equations, input parameters and variables.

Genet growth relies on probabilistic laws and parameters related to basic metabolism, modalities of resource translocation and storage, structural constraints and modalities of elongation *vs*. branching.

Basic metabolism

Resource of the clone depends on plant metabolism and resource strategy:

(L1): $R_C\ (t+1) = R_C\ (t) + \sum_{i=1}^{n} R_i(t) - a\,C\ \ t \in [1;100]$ from an initial condition that corresponds to a unique isolated root of initial biomass $R_C(1) = 1$.

where *i* is the number of a ramet, $R_C(i)$ is the total resource of the clone at the time *t*, r_p is the net resource uptake by one ramet for one time step, $R_C(i)$ is the gain of biomass at time t for ramet I and *C* is the cost of production of new growth units , either a ramet or a connection unit (C_r and C_c respectively). *a* is null if no growth unit is created, one otherwise.

The accumulation of resources for one growth unit was modeled as a logistic law (L2):

(L2) $\frac{dR(t)}{dt} = r_p\, R(t)\left(1 - \frac{R(t)}{r_m}\right)$

where $R(t)$ is the resources available at the time *t*, r_p is the resource uptake by one ramet for one time step and r_m is the maximum resource content for one growth unit. r_m was fixed to 20 for ramet growth units and 10 for connection growth units.

Resource translocation and storage

Resource acquired by a ramet growth unit is shared among all the growth units of an Integrative Physiological Unit (IPU), following (L3):

(L3) $R_{IPU}(r) = \sum_{i=1}^{i=d_r} R(i)$

where d_r is the number of growth units of an IPU and $R(i)$ is the resources available for each growth unit.

Structural constraints

The number of connections produced depends on the activation of buds available. Branching from the parent ramet growth unit corresponds to the production of primary connections. It can occur in six directions (60, 120, 180, 240, 300, 360°) and depends on the number of buds n_i. Branching from other ramets leads to the formation of branch connections (*i.e.* second-

order or third-order connections). It can occur in two directions (60, 300°) and is governed by the number of buds n_b.

The inter-ramet distance depends on the spacer length and its variability, following (L4):

(L4) $D(i,j) = d_o + \mu \qquad \mu \in [0; d_1]$.

where *D(i, j)* is the spacer length at the growth unit of coordinates *(x, y)* among the grid, d_0 is the mean number of connection units for a spacer and μ is a random variable in the range 0 to d_1. d_1 is the maximum number of connection units that can be added to d_0. The maximum length of the spacer is therefore (d_0 + d_1).

Processes of elongation *vs.* branching

A trade-off between elongation and branching processes was implemented in the model following (L5):

(L5) $p_{el/br} = \alpha \qquad \alpha \in [0; 1]$

$if\ p_{el/br} > p_{el/br(0)}$, elongation process,

$if\ p_{el/br} \leq p_{el/br(0}$, branching process

where $p_{el/br}$ is the probability to elongate, α is a random variable in the range 0 to 1 and $p_{el/br(0)}$ is the threshold value for elongation versus branching process.

The probability of elongation of each branch *(p_{el})* was calculated following (L6):

(L6) $p_{el}(b) = \frac{\beta}{(1+E_g G(b))(1+E_l L(b))} \qquad \beta \in [0; 1]$

where p_{el} *(b)* is the probability of elongation of each branch *(b)*, *β* is a random variable in the range 0 to 1, *G(b)* and *L(b)* the generation number and the length of the branch respectively, E_g and E_l the dependence of elongation on the generation number and length of the branch respectively.

The probability of elongation is higher for low generation number (primary branches) and small branches. High values of E_g and E_l increase the weight of, respectively, the generation and the length of a branch, in its probability of elongation.

The probability of branching of each branch *(p_{br})* was calculated following (L7):

(L7) $p_{br}(b) = \gamma \frac{(1+E_l L(b))}{(1+E_g G(b))} \qquad \gamma \in [0; 1], \quad \text{if } G(b) < 3$

$p_{br}(b) = 0 \qquad \text{if } G(b) \geq 3$

where p_{br} *(b)* is the probability of branching of each branch *(b)*, γ is a random variable in the range 0 to 1, *G(b)* and *L(b)* the generation number and the length of the branch respectively, B_g and B_l the dependence of branching on the generation number and length of the branch respectively.

The probability of branching is higher for low generation (primary branches) and long branches. High values of B_g and B_l increase the weight of, respectively, the generation and the length of a branch, in its probability of branching.

The probability of creating a new growth unit at each ramet node along the connection (p_r) was calculated following (L8):

$$p_{br}(r) = \frac{\delta}{(1+B_p D(r))} \qquad \delta \in [0;1] \qquad \text{(L8)}$$

where $p_{br}(r)$ is the probability of branching of each ramet *(r)* along the branch, δ is a random variable in the range 0 to 1, *D(r)* is the distance between the ramet *r* considered and the branch basis, B_p the dependence of the branching process along the branch on the length of the branch.

The probability of branching is high for ramet units situated close to the branch basis (low apical dominance) and low for ramet units situated far away from the branch basis (*i.e.* close to the branch apex, high apical dominance). High values of B_p increase the weight the position of a ramet unit along the branch in probability of branching.

Patterns of defoliation

At the time of a defoliation event, which depends on the frequency of defoliation (*F*), the coordinates *(x',y')*of each defoliated unit depend on the percentage of defoliated cells (*P*) and the mean number of units constituting of the basis cluster of defoliation (*G*).

The impact of defoliation depends on the cell status (ramet unit, connection unit, empty cell). If a defoliated cell is superimposed to a ramet growth unit, this latter lose quantity of biomass proportionally to its biomass at the time of the defoliation (L9):

$$R_{(t+1)} = R_{(t)} \times I \qquad \text{(L9)}$$

where $R_{(t+1)}$ is the biomass of the ramet at the time of the defoliation event, $R_{(t)}$ is the biomass of the ramet just after the defoliation event and *I* the proportion of biomass lost by a defoliated ramet unit.

The updating process

A simulation is initiated by the placement of the parent ramet unit in the center of the hexagonal lattice. Each growth unit *(x,y)* are given a spacer length *D* using the equation (L4). Within each time period, phases are processed in the following order:

1- Defoliation event according to the frequency of defoliation (*F*). If the time step corresponds to a defoliation event, defoliation occurs: equation (L9).

2- Calculation of resource: equations (L2) and (L3)

3- Calculation of the location of the potential new growth unit. Once the event (elongation or branching) is determined (L5), the location of the newly created growth unit depends on the probability (L6) if the event is an elongation and on the probabilities (L7) and (L8) if the event is branching. The cell having the highest probability of becoming a new growth unit is selected.

4- Calculation of the type of the new growth unit depending on the spacer length *D* attributed to the last ram*et al*ong the branch: if the distance from the last ramet is lower than the spacer length, it becomes a connection unit; otherwise a ramet unit is produced.

5- Calculation of the probability of creating the new growth unit. The resource balance is analyzed within the IPU corresponding to the potential placement of the new growth unit. The probability of a new unit *(p_g)* being created depended on the ratio of resources available within the IPU *versus* the cost of producing the grow unit following (L11):

(L10): $p_g = \varepsilon \left[1 + log\left(\frac{R_{IPU}}{c_g}\right)\right]$ with $\varepsilon \in [0;1]$

$c_g = c_r$ or c_c

where p_g is the probability of creating the new growth unit, ε is a random variable in the range 0 to 1, R_{IPU} is the available resources within the IPU and C_r and C_c are the production costs of one ramet and one connection respectively. The growth unit is created if $p_g > p_{g0}$.

If the potential location of a ramet growth unit is already occupied, it is not created.

Conclusion du chapitre 4

Ce dernier chapitre, reposant sur des résultats de modélisation, permet de discuter de l'implication des propriétés clonales dans la réponse au patron spatio-temporel de la défoliation, mais il ouvre également la voie à de nombreuses réflexions.

Tout d'abord, nous avons trouvé que les combinaisons de traits clonaux optimales, *i.e.* permettant de maximiser la performance clonale sont rares. Le plus petit nombre est enregistré sous défoliation homogène, lorsque l'indicateur de performance considéré est la biomasse. Par conséquent, la défoliation homogène ressort comme un filtre fort. D'autre part, bien que l'absence de défoliation soit un filtre plus faible, les combinaisons de traits optimales sous ces deux contrôles homogènes sont similaires, et diffèrent des combinaisons optimales émergeant en conditions de défoliation hétérogène. Les formes clonales optimales en conditions homogènes, défoliées ou non, reposent sur des contraintes structurales décrites expérimentalement chez *E. palustris* (ARTICLE 5). Ce type d'architecture permet de maximiser l'occupation spatiale tout en limitant l'investissement dans le réseau de connexions.

Contrairement à nos résultats empiriques, dans ce travail de modélisation les capacités de stockage ne jouent aucun rôle dans l'optimisation des performances clonales, quel que soit le patron de défoliation. Ce résultat pourrait en partie être lié au paramétrage du modèle quant à la mise en réserves ou à une sévérité de la défoliation trop faible.

Enfin, les combinaisons de traits clonaux optimales sous défoliation hétérogène, sont complexes, notamment car l'expression de certains traits varie selon le paramètre de défoliation considéré (pourcentage, grain, fréquence ou intensité). Cependant, quelles que soient les caractéristiques de la défoliation, les conditions hétérogènes favorisent les traits clonaux permettant la dispersion spatiale des ramets. Cette architecture constituerait une stratégie de répartition du risque de défoliation entre les ramets. Les formes clonales optimales décrites sous défoliation hétérogène restent à exploiter plus en détail, et à confronter à des données empiriques (actuellement en cours d'exploitation).

DISCUSSION GÉNÉRALE

Le pâturage est l'un des principaux modes de gestion des écosystèmes herbacés terrestres par l'Homme (Diaz *et al*. 2007). De ce fait, les relations entre pâturage, végétation et biodiversité font l'objet d'études diverses depuis de nombreuses décennies. Le pâturage est un phénomène complexe s'appliquant aux plantes. La compréhension, voire la prédiction de ses impacts sur les communautés végétales passe par l'analyse des mécanismes impliquant les diverses composantes qui le constituent. La présente étude avait pour objectif de tester l'hypothèse que le pâturage favorise les combinaisons de traits clonaux qui confèrent aux plantes des capacités de résistance, notamment à la défoliation qu'il génère. Plus particulièrement, nous avons cherché à démêler l'importance relative des traits clonaux dans la réponse à la défoliation et à déterminer leur implication dans la résistance des plantes au pâturage. A l'issue de ce travail, la discussion des résultats s'articule autour des questions suivantes :

1) Quel est l'impact du pâturage sur la végétation, notamment sur ses caractéristiques clonales ?
2) Quelles sont les réponses architecturales à la défoliation ? L'architecture clonale est-elle un bon indicateur de la résistance au pâturage ?
3) Les propriétés physiologiques associées à la clonalité, notamment la mise en place de réserves, sont-elles impliquées dans la tolérance à la défoliation et la résistance au pâturage ?
4) La clonalité permet-elle une réponse efficace à la défoliation hétérogène ?

Enfin, après avoir évoqué les limites de cette étude, nous aborderons les perspectives ouvertes par ce travail.

1. Le pâturage comme filtre environnemental

1.1. Un filtre environnemental secondaire

Le pâturage impacte la végétation, non seulement en termes de richesse, diversité et composition spécifiques, mais également d'un point de vue fonctionnel. Il agit comme un filtre environnemental, favorisant les traits qui permettent aux plantes de se maintenir, c'est-à-dire de croître et de se régénérer, dans les conditions qu'il génère (traits de réponse Lavorel & Garnier 2002). Nos travaux ont montré que l'impact du pâturage sur les traits clonaux est modulé par le régime d'inondation (ARTICLE 1). Plusieurs études ont montré que le régime d'inondation influence les effets du pâturage sur la structure et la diversité de la végétation (Oesterheld & McNaughton 1991, Insausti *et al.* 1999, Jutila 1999, Mesléard *et al.* 1999). En effet, l'inondation favorise les espèces capables de tolérer des conditions d'immersion, de

faible incidence lumineuse ou d'hypoxie, voire d'anoxie par exemple (Blom & Voesenek 1996). Dans la zone d'étude du Marais Poitevin, le régime d'inondation, en lien avec le micro-relief, conduit à la discrimination de trois communautés végétales (hygrophile, méso-hygrophile et mésophile) différant tant d'un point de vue spécifique que fonctionnel et plus particulièrement clonal. Le régime de pâturage n'influence pas la composition clonale dans la communauté hygrophile, certainement parce que l'inondation et le pâturage tendent à favoriser des traits similaires (ARTICLE 1). A l'inverse, les traits clonaux des communautés méso-hygrophile et mésophile se sont avérés sensibles au régime de pâturage.

Nos résultats confirment donc, sur les traits clonaux, des observations déjà réalisées sur d'autres traits, le plus souvent morphologiques : bien que quelques traits émergent comme de bons indicateurs de la réponse au pâturage, ces observations ne sont généralement fiables qu'à échelle locale. Outre le régime d'inondation, le climat, la productivité du site ou encore l'historique du pâturage agissent comme des filtres environnementaux contraignant la composition spécifique et fonctionnelle de la végétation et, par conséquent la réponse au pâturage (*e.g.* Pakeman 2004, de Bello *et al*. 2005, Diaz *et al*. 2007).

Il n'existe donc pas une mais des réponses clonales au pâturage qui dépendent de la communauté végétale étudiée. Ces observations nous ont amenés à focaliser nos travaux principalement sur la végétation mésophile, car elle présente une composition clonale diversifiée et une réponse au pâturage marquée.

1.2. Le pâturage générateur d'hétérogénéité spatiale à échelle grossière

Dans les prairies étudiées, le pâturage génère une mosaïque de patches de structure, composition et diversité végétales différentes, à l'échelle de plusieurs dizaines voire plusieurs centaines de mètres (Loucougaray *et al*. 2004, Rossignol *et al*. 2006). Nos résultats ont confirmé l'existence d'une hétérogénéité grossière (de plusieurs mètres à plusieurs dizaines de mètres) de la structure de la végétation sous pâturage modéré, tandis que le pâturage intense tend à homogénéiser le couvert végétal (ARTICLE 2). En effet, lorsque l'intensité de pâturage augmente, notamment du fait d'un plus grand nombre d'herbivores, la quantité de fourrage par animal diminue, obligeant ces derniers à exploiter la végétation de manière plus complète, voire à s'alimenter d'espèces de qualité nutritionnelle moindre (Weber *et al*. 1998). Par ailleurs, nos résultats montrent l'existence d'une hétérogénéité fine (patches de 0,2 à 0,5 m de diamètre) quel que soit le régime de pâturage, et y compris en conditions non pâturées. Cette hétérogénéité serait donc intrinsèque à la végétation, notamment liée à l'identité des espèces présentes et pourrait également traduire une hétérogénéité fine des conditions

environnementales (Rietkerk *et al*. 2000, Adler *et al*. 2001). Le pâturage modéré tend à augmenter cette hétérogénéité de la hauteur du couvert végétal. Néanmoins, cette observation n'est pas généralisable à l'ensemble des relevés réalisés sous pâturage modéré. Par conséquent, il ressort de cette étude que le pâturage bovin tend à générer une hétérogénéité grossière, ne pouvant être perçue à l'échelle de la plante clonale. En effet, bien que certains fragments clonaux puissent couvrir des surfaces de plusieurs centaines de m², voire plusieurs hectares, ces cas sont des exceptions plus que la norme. En outre, l'intégration physiologique extensive se limite généralement à quelques mètres (D'Hertefeld & Jónsdóttir 1999, D'Hertefeld & Falkengren-Grerup 2002). Wijesinghe & Hutchings (1997) ont montré que la réponse de genets de *Glechoma hederacea* à l'hétérogénéité spatiale dépend de son échelle, l'optimum correspondant à des patches de 25 × 25 cm. La perception de conditions environnementales hétérogènes par des plantes clonales herbacées clonales est donc restreinte à des échelles fines, généralement inférieures à 1 m². Dans les prairies étudiées, l'influence de la défoliation induite par le pâturage est, par conséquent, principalement liée à son intensité (hauteur de coupe ou proportion de tissus retirée) ou sa fréquence (pas de temps entre deux évènements de défoliation successifs).

Par la suite, nous nous sommes attachés à décrire l'impact de la défoliation générée par le pâturage bovin sur l'expression des traits clonaux. Celle-ci étant homogène à l'échelle de la plante clonale, nous pouvions nous attendre à ce que les formes clonales sélectionnées investissent dans des mécanismes (i) d'évitement spatial, (ii) d'évitement temporel ou (iii) de tolérance (Tableau III). Nous avons donc cherché à identifier les mécanismes de résistance à la défoliation générée par le pâturage et les traits clonaux impliqués, en nous focalisant (i) sur la morphologie et l'architecture clonale et (ii) sur les traits physiologiques (stockage et intégration clonale).

Tableau III – Comparaison des traits clonaux observés en réponse au pâturage et/ou à la défoliation avec les hypothèses. Type de données : relevés de terrain couplées aux traits CLO-PLA3 (*in situ* + CloPla3) ou au données expérimentales (*in situ* + exp.), données expérimentales (Exp.), résultats de la modélisation (Simulations). La colonne « Ref » renvoie au numéro des articles où sont présentés ces résultats.

	Mécanisme de résistance	Traits clonaux attendus	Traits clonaux observés	Données	Ref
Défoliation homogène	**Evitement**				
	Evitement spatial	- Organes clonaux porteur d'une banque de bourgeons souterraine (bulbes, tubercules, racines, rhizomes) ou proche de la surface du sol (stolons rampants)	Formes clonales stolonifères et cespiteuses.....	*in situ* + CloPla3	1 - 2
			Banque de bourgeons aérienne…….	*in situ* + CloPla3	1 - 2
	Evitement temporel	- Ramets annuels à développement précoce ou automnal……..	Ramets pérennes……...	*in situ* + CloPla3	1 - 2
		- Organes clonaux pérennes…….....	Organes clonaux annuels…….....	*in situ* + CloPla3	1 - 2
	Tolérance				
	Croissance compensatoire	- Organes de stockage : ~souterrains (principalement)……	↘ investissement dans les organes clonaux souterrains……	*in situ* + exp.	3
		~aériens (dans une moindre mesure)…….	Formes clonales stolonifères et cespiteuses.....	*in situ* + CloPla3	1 - 2
		- Stockage dans la base des tiges…….	Stockage dans la base des tiges……	Exp.	7
	Régénération végétative	- Fort taux de multiplication clonale……	↘ taux de multiplication clonale…….....	*in situ* + CloPla3	1 - 2
			↘ ou → de la production de ramets……..	Exp.	4 - 5
		- Fort taux de ramification (architecture de type phalange)…….	↘ ou → de la production de connexions……...	Exp.	4 - 5
				Simulations	8
			↘ ou → de la longueur des connexions et des distances inter-ramets……..	Exp.	4 - 5
Défoliation hétérogène	**Evitement spatial**	- Connexions et distance inter-ramets longues (architecture de type guérilla)	→ de la longueur des connexions…….....	Simulations	8
			↗ des distances inter-ramets…….	Simulations	8
	Tolérance	- Intégration physiologique extensive……..	→ distance d'intégration physiologique……..	Simulations	8
		- Spécialisation des ramets non défoliés dans l'acquisition des ressources……..	En cours d'analyse…….……..	Exp.	

2. Réponses morphologiques à la défoliation : l'architecture clonale est peu impliquée dans la résistance au pâturage

2.1. Impact du pâturage sur le type d'organe clonal

Contrairement à nos attentes (Tableau III), le pâturage favorise les espèces produisant des organes clonaux aériens et à durée de vie courte, notamment les formes clonales stolonifères et cespiteuses (ARTICLES 1 ET 2). A l'inverse les formes clonales rhizomateuses sont plus abondantes en conditions non pâturées (ARTICLES 1 ET 2). Dans les prairies de notre site d'étude, huit espèces clonales pérennes dominent la végétation (ARTICLE 3). Parmi ces espèces, les quatre espèces présentes uniquement en conditions pâturées sont cespiteuses (*C. cristatus*, *H. secalinum*, *L. perenne* et *P. trivialis* ; ARTICLE 3). L'avantage des stolons à courte durée de vie, ainsi que des rhizomes courts caractéristiques des formes cespiteuses pourrait se trouver dans le faible coût associé à leur production et à leur maintenance (Grace 1993, de Kroon & Schieving 1990, van Groenendael *et al*. 1996). Les pertes de biomasse et la croissance compensatoire généralement induite par la défoliation représentent un coût supplémentaire pour la plante (van der Meijden *et al*. 1988), pouvant favoriser les formes clonales permettant la production de ramets tout en limitant les investissements associés à la croissance clonale. A l'inverse, sur les quatre espèces clonales présentes tant en conditions pâturées que non pâturées, deux sont exclusivement rhizomateuses (*C. divisa* et *J. gerardii*), une est à la fois rhizomateuse et cespiteuse (*E. repens*) et une à la fois stolonifère et cespiteuse (*A. stolonifera* ; ARTICLE 3). L'expansion latérale sur de longues distances serait une propriété associée à la compétitivité de l'espèce (Grime 1977). Les réserves potentiellement stockées dans les connexions pourraient être un atout supplémentaire, permettant la croissance et la multiplication clonale en environnement compétitif. Nous n'avons cependant pas mis en évidence de relation entre la distance de propagation clonale et la résistance au pâturage chez les huit espèces étudiées (ARTICLE 3). Ce résultat est probablement dû en partie à la durée de l'expérimentation à l'issue de laquelle ont été mesurés les traits clonaux. En effet, l'expérimentation n'a été menée que sur quelques mois, amplifiant les différences de taux d'expansion, notamment entre *A. stolonifera* et *E. repens* d'une part et *C. divisa* et *J. gerardii* d'autre part (observations personnelles). Chez ces deux Cyperaceae, une expérimentation d'une durée d'un an a en effet permis de constater l'existence d'une période de croissance relativement lente, suivie d'une phase d'expansion beaucoup plus rapide (projet en cours d'analyse).

2.2. Impact du pâturage sur la morphologie et l'architecture clonale

En accord avec les premières observations réalisées sur les types d'organes clonaux, nos résultats mettent en évidence un impact du pâturage sur l'expansion latérale et la position de la banque de bourgeons végétatifs. Contrairement à nos attentes, le pâturage ne favorise cependant pas les capacités de régénération végétative (taux de multiplication clonale, *i.e.* nombre de ramets produits). Au contraire, le taux de multiplication clonale tend à être plus faible en conditions pâturées qu'en absence de pâturage (ARTICLES 1 ET 2).

Le pâturage semble limiter l'expansion latérale. Ceci s'explique certainement par le fait qu'*in situ*, la défoliation générée par le pâturage est homogène à l'échelle de plusieurs centaines de cm^2 voire plusieurs m^2. D'après nos hypothèses, une propagation latérale sur de grandes distances (*i.e.* de plusieurs décimètres à quelques mètres) serait avantageuse uniquement en conditions de défoliation hétérogène dans lesquelles elle permettrait une exploration efficace de l'espace, voire une fuite hors des zones défoliées (Tableau III). A l'inverse, un investissement dans l'élongation des connexions en conditions homogènes ne permettrait pas au fragment clonal d'expérimenter des conditions contrastées de défoliation. Dans ce cas, les coûts associés à la production et au maintien des connexions ne seraient pas contrebalancés par l'avantage de la dispersion, rendant la propagation clonale désavantageuse.

Nous avons montré que la taille de la banque de bourgeons aérienne était importante (ARTICLES 1 ET 2). La présence d'une banque de bourgeons végétatifs au-dessus de la surface du sol pourrait être avantageuse en prairies pâturées car elle permettrait une régénération végétative efficace suite à la défoliation (Bellingham & Sparrow 2000, Lasso *et al*. 2009, Klimešová & Klimeš 2003, Klimešová & Klimeš 2007). Nous avons cependant mis en évidence que le taux de multiplication clonale des espèces présentes en conditions pâturées est inférieur à celui des espèces dominantes en absence de pâturage. Ce décalage entre la taille réelle de la banque de bourgeons et le taux de multiplication clonale (production de ramets) pourrait être dû à la dormance des bourgeons chez les espèces étudiées.

Ces résultats issus d'observations *in situ* et d'informations collectées dans la base de données CLO-PLA3 ne sont cependant pas confirmés lorsque les traits clonaux sont mesurés expérimentalement sur les plantes collectées *in situ* et cultivées en jardin expérimental (ARTICLE 3). En effet, selon cette étude, seules la hauteur végétative et la biomasse des organes clonaux souterrains sont négativement corrélées à la résistance des espèces au pâturage. La hauteur des plantes est en effet l'un des principaux traits indicateur de la résistance au pâturage, une petite stature étant associée à l'évitement spatial, tandis qu'une taille importante permet aux plantes d'être compétitives pour la lumière et est avantageuse en

conditions non perturbées (Grime 1977, Briske 1996, Diaz *et al*. 2007). De la même manière, un investissement important dans les organes souterrains serait associé à la compétitivité de l'espèce en conditions non pâturées. Néanmoins, au cours de cette expérience, la mesure des traits a été réalisée sur des plantes cultivées en conditions contrôlées et non perturbées. Des mesures de traits *in situ*, permettant de prendre en compte les variations intra-spécifiques et en présence de compétition seraient nécessaires pour vérifier ces résultats.

En croisant les patrons d'abondance des espèces *in situ* et leurs traits clonaux issus de la base de données CLO-PLA3 ou mesurés en conditions non perturbées, ces premiers résultats ont permis de décrire les traits clonaux potentiels des communautés végétales sous divers régimes de pâturage. Par la suite, nous avons cherché à caractériser la plasticité de ces traits clonaux en réponse à la défoliation. En effet, une plasticité phénotypique importante en réponse à la défoliation pourrait aboutir à l'expression de traits contrastés sous divers régimes de pâturage, malgré des traits potentiels identiques.

2.3. Réponse de l'architecture clonale à la défoliation : des patrons variés

2.3.1. La réponse architecturale à la défoliation ne détermine pas la réponse au pâturage

Nos résultats ont permis de montrer que seules les réponses à la défoliation de la hauteur végétative et de la biomasse des organes clonaux souterrains sont significativement liées à la résistance des espèces étudiées au pâturage. La diminution de ces deux traits en réponse à la coupe est moindre chez les espèces les plus résistantes au pâturage (*i.e.* dont l'abondance augmente avec le régime de pâturage) que chez les espèces les moins résistantes (*i.e.* dont l'abondance diminue avec un régime de pâturage croissant). Cependant, les différences de résistance au pâturage ne peuvent être expliquées par une réponse plastique des traits d'architecture clonale. Contrairement à nos attentes (Tableau III), ni l'augmentation de la production de ramets, ni une ramification accrue n'ont été relevées chez les espèces les plus résistantes au pâturage (ARTICLE 3). L'absence de relation entre la résistance au pâturage et la réponse architecturale à la défoliation peut avoir plusieurs causes. (i) La réponse architecturale à la défoliation est similaire entre les espèces, quelle que soit leur résistance au pâturage. (ii) Il n'existe pas une mais des réponses à la défoliation qui permettent aux plantes de se maintenir en conditions pâturées. (iii) Notamment, les réponses à la défoliation peuvent être limitées par des contraintes structurales propres à l'espèce. Nous avons donc cherché à

caractériser les réponses architecturales à la défoliation chez plusieurs espèces de formes clonales variées.

2.3.2. La réponse architecturale à la défoliation est limitée par des contraintes structurales spécifiques

Nous avons mis en évidence que la réponse architecturale à la défoliation ne dépend pas du type de connexions (*e.g.* stolons *vs*. rhizomes ; ARTICLE 4). Nous avons en effet détecté des réponses architecturales similaires chez des espèces stolonifères et rhizomateuses. Ces résultats vont à l'encontre d'études antérieures ayant démontré une plasticité plus importante des stolons que des rhizomes en réponse aux conditions environnementales (Dong & de Kroon 1994, Dong & Pierdominici 1995).

Il semblerait donc que la réponse architecturale à la coupe dépende de contraintes structurales plus subtiles que la simple nature des connexions (*structural blue-print sensu* Huber *et al.* 1999). Par exemple, le nombre de méristèmes axillaires est un facteur limitant le nombre de ramifications pouvant être potentiellement produites (Huber & During 2001). Ainsi, la défoliation des deux espèces monopodiales (*T. fragiferum* et *T. repens*), chez lesquelles le nombre de méristèmes par ramet est limité, a généré une diminution du nombre de connexions plus importante que chez les espèces sympodiales étudiées lors de la même expérience (ARTICLE 4). L'importance des contraintes structurales a été confirmée par les réponses architecturales à la défoliation observées chez deux Cypéracées rhizomateuses, *C. divisa* et *E. palustris* (ARTICLE 5). Chez *C. divisa*, la réponse architecturale à la défoliation s'est traduite par la réduction du nombre de ramifications et l'augmentation de l'angle de ramification, tandis qu'aucun changement architectural n'a eu lieu chez *E. palustris*. Ces différences de réponse à la défoliation semblent s'expliquer par des contraintes structurales contrastées. En effet, tandis que le réseau de rhizomes de *C. divisa* repose essentiellement sur la ramification, les fragments clonaux d'*E. palustris* produisent de nombreux rhizomes primaires mais se ramifient peu. Par conséquent, il semblerait que les ressources nécessaires à la croissance compensatoire ont été utilisées aux dépens de la production et de l'élongation de ramifications (Meyer & Schmid, 1999), affectant principalement les genets de *C. divisa*.

D'après nos données de modélisation, les contraintes structurales permettant de maximiser la biomasse du genet et le nombre de ramets en absence de défoliation et sous défoliation homogène sont similaires. Elles sont caractérisées par un grand nombre de bourgeons primaires, autorisant la production d'un grand nombre de connexions primaires, et des distances inter-ramets petites (ARTICLE 8). Ces propriétés architecturales correspondent à

celles décrites chez *E. palustris* (ARTICLE 5). Ce type d'architecture, déjà décrit chez d'autres espèces clonales (Meyer & Schmid, 1999, Brun *et al*. 2007) permettrait de maximiser l'occupation de l'espace, tout en limitant l'investissement dans le réseau de connexions (Smith & Palmer 1976, Meyer & Schmid 1999). En effet, en conditions homogènes où l'exploration spatiale ne permet pas au genet de rencontrer des conditions contrastées, un investissement dans la production de ramets plutôt que dans celle de connexions (via de faibles distances inter-ramets) semble le plus avantageux. A l'inverse, en conditions de défoliation hétérogène, quels qu'en soient le pourcentage, le grain, la fréquence ou l'intensité, des architectures caractérisées par de longues distances inter-ramets seraient optimales. En effet, en environnement hétérogène, la prospection spatiale et de grandes distances inter-ramets permettraient de disperser le risque de défoliation entre les ramets d'un même genet (Piqueras 1999).

2.3.3. Conclusion

D'après nos résultats, la défoliation induit une baisse ou, au mieux, un maintien de l'investissement dans la propagation et la multiplication clonale, comme le traduisent la stabilité ou la diminution des traits architecturaux (longueur moyenne des connexions, distance inter-ramets, nombre de connections), du nombre de ramets et/ou de la surface occupée par le genet (ARTICLES 4 et 5). Un investissement limité dans la propagation clonale, une diminution de la viabilité des bourgeons et du nombre de ramets en réponse à la coupe ont déjà été montrés (*e.g.* Bullock *et al*. 1994, Newton & Hay 1996, Moen *et al*. 1999, Wang *et al*. 2004, Henry *et al*. 2007) suggérant que les coûts liés à la perte de biomasse et à la croissance compensatoire consécutive à la défoliation limiteraient l'investissement dans les structures clonales.

L'effet majeur de la défoliation sur l'architecture clonale semble donc être une baisse de l'investissement dans le développement du réseau de connexions (élongation et/ou ramification). Des différences structurales (*structural blue-print*) entre les espèces modèlent cet effet pour aboutir à une diversité de réponses architecturales à la défoliation. Par conséquent, il semble qu'une diversité d'architectures clonales puisse s'exprimer en conditions pâturées.

3. Réponses physiologiques à la défoliation : le stockage comme mécanisme de résistance au pâturage

3.1. Le pâturage ne favorise pas les organes spécialisés dans le stockage

Aucune espèce présente dans notre site d'étude ne produit de tubercules ou de bulbes. Comme nous l'avons vu précédemment et contrairement à nos hypothèses (Tableau III), le pâturage favorise les formes clonales stolonifères, tandis que les formes rhizomateuses dominent la végétation en l'absence de pâturage (ARTICLES 1 ET 2). Ainsi, la biomasse des organes clonaux souterrains est négativement corrélée à la résistance des espèces au pâturage : les espèces les plus résistantes au pâturage sont celles qui allouent le moins de biomasse aux organes clonaux souterrains, et chez lesquelles la défoliation affecte le moins cette biomasse (ARTICLE 3). Or les rhizomes sont généralement considérés comme des organes particulièrement efficaces dans le stockage de ressources (Dong & de Kroon 1994, Dong & Pierdominici 1995). La mise en place de réserves ne serait donc pas associée à la résistance au pâturage mais favoriserait plutôt à la fois l'occupation de l'espace souterrain et le stockage de ressources, conférant aux plantes une importante aptitude compétitive (Grime 1977, Barney *et al*. 2005, 2009).

Selon des données issues du modèle, la proportion de ressources allouées aux connexions et, par conséquent, protégées de la défoliation (seuls les ramets pouvant être endommagés), n'a aucune implication dans la réponse à la coupe, quelles que soient ses caractéristiques spatiales, sa fréquence ou son intensité (ARTICLE 8). D'après ces résultats de modélisation, les capacités de stockage n'interviendraient donc pas dans la réponse au pâturage. Cependant, outre les organes souterrains, les autres structures clonales peuvent être impliquées dans le stockage de réserves (Suzuki & Stuefer 1999, Stuefer & Huber 1999). La base des tiges, notamment chez les Poaceae, a également une fonction d'organe de stockage. Du fait de leur proximité avec les tissus endommagés lors de la défoliation, les réserves accumulées dans ces tissus pourraient s'avérer les plus rapidement mobilisées et, par conséquent, les plus importantes dans la réponse à la coupe (Morvan-Bertrand *et al.* 2001).

En outre, diverses substances peuvent être mises en réserve. Bien que la biomasse soit régulièrement considérée comme un indicateur de la quantité de réserves (Cheplick & Gutierrez 2000, van der Meijden *et al*. 2000), celle-ci s'avère en réalité peu précise, puisqu'elle comprend également des composés structuraux (*i.e.* intervenant dans la construction des tissus et organes de la plante, *e.g.* hemicellulose) peu mobilisbles (Chapin *et al*. 1990). Il convient donc de s'interroger non plus seulement sur la quantité, mais aussi sur la qualité des substances contenues dans les tissus végétaux.

3.2. Le pâturage favorise le stockage dans la base des tiges

Suite à un évènement de défoliation, la remobilisation des stocks de ressources, principalement carbonées et azotées, favoriserait la repousse après défoliation, en permettant une reconstitution rapide de tissus photosynthétiques fonctionnels. En effet, deux phases de croissance compensatoire consécutive à un évènement de défoliation, ont été discriminées. La première phase, relativement courte (de quelques heures à quelques jours, Richards 1993), repose sur une remobilisation des substances mises en réserves, permettant la construction de nouveaux tissus photosynthétiques. La seconde phase correspond à la reprise de l'activité photosynthétique et de l'absorption racinaire (revue bibliographique, ARTICLE 6). Par conséquent, plus la phase d'utilisation des réserves est efficace (pools de réserves importants et/ou retranslocation et mobilisation rapides), plus la plante pourra compenser les pertes de tissus et être compétitivement supérieure aux plantes voisines suite à la défoliation.

Nos résultats ont confirmé la relation entre les stocks de substances carbonées (hydrates de carbone non structuraux ou TNC[8]) et plus particulièrement de fructanes, contenus dans la base des tiges et la réponse au pâturage (ARTICLE 7). Chez la plupart des Poaceae des climats tempérés, telles que les six espèces étudiées, les fructanes constituent le principal TNC de réserve dans les organes végétatifs (Pollock & Cairns 1991, Scofield *et al*. 2009). En premier lieu, nous avons montré des variations interspécifiques des stocks de fructanes et de saccharose contenus dans la base des tiges juste avant la saison de pâturage. Les quantités stockées se sont avérées positivement liées à la résistance des espèces au pâturage. En outre, toutes espèces confondues, les plantes se développant sous un régime de pâturage intense présentent des stocks de fructanes plus importants que les plantes collectées sous un régime de pâturage modéré. Il ressort donc de ces mesures que les stocks de TNC seraient impliqués dans la résistance des plantes au pâturage, à la fois via des variations interspécifiques et des variations intra-spécifiques en réponse au régime de pâturage. Les capacités de stockage de réserves dans la base des tiges seraient donc avantageuses en prairies pâturées.

[8] TNC (terme anglais) : Total Nonstructural Carbohydrates.

3.3. Travaux en cours

3.3.1. Quelle est l'importance des réserves stockées dans les rhizomes ?

L'expérience présentée dans l'ARTICLE 7 s'est focalisée sur les réserves stockées dans la base des tiges. Cependant, nous avons montré une relation négative entre la résistance des espèces au pâturage et la biomasse de leurs organes clonaux souterrains. Les espèces favorisées par le pâturage allouent peu de biomasse à ces organes souterrains, tandis que la plupart des espèces dominantes en conditions non pâturées sont rhizomateuses. Outre leur fonction de propagation, les rhizomes servent généralement de structures de stockage (Dong & de Kroon 1994, Dong & Pierdominici 1995, Suzuki & Stuefer 1999). Quel est donc le rôle de ces rhizomes dans le stockage de réserves et dans la réponse des plantes au pâturage ? Contrairement aux réserves stockées dans la base des tiges, dont la proximité avec les tissus endommagés par défoliation pourrait en faciliter la remobilisation, les réserves stockées dans les rhizomes pourraient être plus lentes à remobiliser. Ces réserves, au lieu de permettre une réponse efficace à la défoliation seraient donc plutôt impliquées dans la compétitivité des plantes. La mobilisation des réserves pour soutenir la croissance des ramets juvéniles permettrait la production de descendants sous des canopées denses où l'établissement par les graines est peu probable. D'une part en permettant l'expansion latérale et l'occupation de l'espace souterrain et, d'autre part, en soutenant la production de descendants, les rhizomes constitueraient donc des organes avantageux en conditions non pâturées et compétitives.

Au cours d'une expérience centrée sur trois espèces rhizomateuses présentes tout au long du gradient de pâturage, nous avons donc cherché à caractériser le type et la quantité de TNC de réserves contenue dans les rhizomes et à les comparer aux teneurs contenues dans la base des tiges, et ce sous trois régimes de pâturage (absence, pâturage modéré et intense). Ces résultats, en cours d'analyse n'ont pu être présentés dans le présent manuscrit.

3.3.2. Clonalité et effet mémoire

L'herbivorie appliquée à une plante peut avoir des conséquences sur les graines qu'elle produit et, par conséquent, sur ses descendants (Agrawal 2001). Cet effet maternel correspond à la transmission de caractères induits par l'environnement. Chez les plantes clonales, les ramets sont issus de divisions mitotiques et peuvent rester connectés physiquement ou physiologiquement aux ramets parents. En permettant le mouvement de substances au sein du fragment clonal ainsi que le stockage de réserves, l'intégration clonale jouerait un rôle important dans d'effet mémoire ('*carry-over effect*'). En effet, les conditions

environnementales peuvent influencer la taille du fragment clonal, la quantité de substances stockées, la synthèse de composés secondaires ou encore l'expression des gènes et sont donc susceptibles d'influencer le développement de plusieurs générations de ramets (Schwaegerle *et al*. 2000). Si le principal facteur intergénérationnel est la taille des ramets parents, les autres facteurs évoqués ci-dessus semblent également jouer un rôle significatif (Schwaegerle *et al*. 2000). En prairies pâturées, cet effet mémoire peut jouer un rôle crucial : la pression de pâturage, notamment la défoliation expérimentée par un fragment clonal est en effet susceptible de moduler la croissance et la reproduction des ramets l'année suivante.

Au cours d'une expérience en jardin, nous avons donc cherché à identifier l'impact de la défoliation appliquée à des genets sur leur croissance et leur reproduction au cours de la saison de végétation.

4. Clonalité et défoliation hétérogène à l'échelle du fragment clonal

Nos travaux ont démontré que la défoliation générée par le pâturage bovin est hétérogène à une échelle supérieure à l'échelle de perception supposée du genet et, par conséquent, perçue comme homogène par celui-ci (ARTICLE 2). Dans un premier temps, nous ne nous sommes pas attachés à étudier les potentiels avantages des traits et propriétés clonales dans la réponse à la défoliation hétérogène. Cependant, les plantes clonales peuvent être soumises à d'autres herbivores, tels que les micro-mammifères ou les invertébrés. De par leur taille, leurs effets à l'échelle du genet sont probablement hétérogènes. Notre étude de modélisation a pris en compte cette hétérogénéité. Nous avons ainsi découvert que la défoliation hétérogène devrait favoriser la dispersion spatiale des ramets (grandes distances inter-ramets) certainement car cela permettait de répartir le risque de défoliation entre ceux-ci. De plus, divers traits clonaux, notamment la distance de partage des ressources (intégration physiologique), semblent impliqués de manière complexe dans la réponse à la défoliation hétérogène, dépendante de la composante de défoliation (pourcentage, grain, fréquence, intensité ; ARTICLE 8). Bien qu'aucune plasticité phénotypique n'ait été intégrée dans le modèle, celui-ci démontre l'importance de la dispersion clonale et de l'intégration physiologique dans la réponse à la défoliation hétérogène.

Afin d'étudier les réponses de fragments clonaux soumis à la défoliation hétérogène, et notamment pour vérifier le rôle de la dispersion des ramets et de l'intégration physiologique, nous avons réalisé une expérimentation en jardin, dont les résultats n'ont pas encore été analysés. D'après les résultats de la modélisation, nous pouvons nous attendre à observer une dispersion des ramets accrue en réponse à la défoliation hétérogène. La plasticité

architecturale intra-fragment clonale semble, quant à elle, peu probable. En effet, bien que de nombreuses études se soient attachées à décrire la plasticité architecturale au sein d'un même fragment clonal en conditions de ressources hétérogènes, seulement quelques unes ont effectivement réussi à la démontrer (pour revue, voir de Kroon & Hutchings 1995). Comme nous l'avons vu précédemment, des contraintes structurales limitent l'architecture clonale et sont donc susceptibles de limiter sa plasticité. Cette plasticité architecturale reposerait sur l'activation de méristèmes suite à la perception des stimulii environnementaux par les ramets (Dong 1993, Huber *et al*. 1999). Ce mécanisme nécessite donc que le fragment clonal ait une information fiable de son environnement au moment de son développement. Contrastant avec ce pré-requis, la défoliation s'applique à des ramets portés par des réseaux de connexions déjà développés. Ainsi, bien que la défoliation puisse activer les bourgeons axillaires, amplifiant les processus de ramification au niveau des ramets coupés, il ne permet pas au genet d'ajuster les distances inter-ramets. Les premières observations issues de cette expérience tendent à confirmer l'absence de plasticité architecturale intra-genet en réponse à la défoliation hétérogène.

La réponse à l'échelle du ramet semble plus probable. Ainsi, les ramets défoliés sont susceptibles de présenter des caractéristiques morphologiques (taille, surface des feuilles), physiologiques (stocks de réserves, substances de défense) ou régénératives (activation de bourgeons floraux ou végétatifs) différentes des ramets non coupés, notamment en relation avec leur croissance compensatoire. Néanmoins, les effets de l'intégration physiologique pourraient s'opposer à cette spécialisation des ramets et permettre au genet de moyenner les effets d'une défoliation hétérogène (Harnett 1989). En effet, le transport de ressources permet aux ramets intacts de soutenir la reprise de croissance des ramets endommagés (Jónsdóttir & Callaghan 1989, Hutchings 1999), tandis que la translocation de messagers induit une réponse systémique et la mise en place de mécanismes de défense par plusieurs ramets (Gómez & Stuefer 2006). Nos données de modélisation suggèrent en effet une implication complexe de l'intégration physiologique en fonction des paramètres de défoliation (ARTICLE 8).

5. Limites de l'étude

L'une des principales limites des travaux réalisés au cours de cette thèse réside dans la difficulté voire l'impossibilité de mesurer les traits clonaux *in situ* (Weiher *et al*. 1999, Klimešovà & de Bello 2009). La compilation des données de composition spécifique récoltées sur le terrain et des traits clonaux collectés dans la base de données CLO-PLA3 (Klimešová & Klimeš 2008) offre une première opportunité de décrire les formes clonales sous différents

régimes de pâturage. Cependant, ces informations se basent sur les traits potentiellement exprimés par les plantes, mais ne permettent pas de prendre en compte la réponse effective des plantes au pâturage et, plus particulièrement, l'impact du régime de pâturage sur les traits clonaux. En effet, ces méthodes ne permettent pas de prendre en compte la variabilité intra-spécifique, qu'elle soit liée à des adaptations locales (sélection des traits les mieux adaptés au régime de pâturage local aboutissant à des populations génétiquement différenciées ; Sultan & Spencer 2002, Lenssen *et al*. 2004), à la plasticité phénotypique (capacité d'un génotype d'exprimer différents phénotypes en fonction des conditions environnementales ; Bradshaw 1965) et/ou à des effets mémoires (transmission aux générations suivantes de caractères phénotypiques induits par l'environnement, Schwaegerle *et al*. 2000).

Des mesures de traits clonaux *in situ*, telles que celles réalisées pour mesurer l'impact du pâturage sur les réserves carbonées (ARTICLE 7), seraient les méthodes les mieux adaptées pour décrire, non plus les traits potentiels, mais les traits réellement exprimés par les plantes sous divers régimes de pâturage. La culture et la mesure des traits clonaux de plantes récoltées sous différents régimes de pâturage et en présence de compétiteurs permettraient en outre de déterminer l'importance des adaptations locales, bien que cette méthode ne permette pas de faire la distinction entre différenciation génétique et effet mémoire. Enfin des expériences de transplantations pourraient être réalisées dans l'optique de décrire la plasticité phénotypique en réponse au pâturage.

6. Conclusions et perspectives

La croissance clonale chez les plantes est un phénomène complexe, associé à des propriétés singulières. Il n'existe donc pas une, mais des clonalités, dont les caractéristiques dépendent des conditions environnementales (Klimeš *et al*. 1997). Ainsi, les plantes clonales dominent la végétation prairiale du site étudié au cours de cette thèse, mais les formes clonales varient en fonction du régime de pâturage. Nous avons donc cherché à caractériser les réponses des traits clonaux à la défoliation et à déterminer leur implication dans la résistance des plantes au pâturage. Bien que nous ayons tenté d'explorer l'éventail de pistes s'ouvrant à nous, il nous reste encore de nombreuses voies à explorer, comme le montrent notamment les diverses expériences en cours d'analyse.

A l'issue de ce travail, il semble cependant que les coûts liés aux pertes de biomasse, à la croissance compensatoire et, certainement, aux blessures associées à la défoliation, jouent un rôle majeur dans l'expression des traits clonaux. En effet, bien que l'architecture clonale soit contrainte par les propriétés structurales spécifiques, la défoliation provoque au mieux un

maintien, mais dans la plupart des cas une baisse de l'investissement du genet dans son réseau de connexions, voire même dans la production de ramets. Les formes clonales les mieux adaptées à la défoliation seraient celles qui maximisent l'occupation de l'espace tout en limitant l'allocation de biomasse aux structures clonales. En prairies pâturées la compaction du sol par le piétinement pourrait s'ajouter à ce processus freinant l'expansion latérale (Schmid & Bazzaz 1990). Ces formes clonales caractérisées par des connexions relativement courtes, une plasticité architecturale faible et des capacités de stockage importantes, peuvent être comparées aux stratégies consolidatrices décrites par de Kroon & Schieving (1990) qui doivent leur nom à leur capacité d'occupation de l'espace et de persistance locale du fragment clonal.

Les formes clonales investissant extensivement dans l'élongation des connexions ont été suggérées comme avantageuses en environnements perturbés, car elles permettraient la colonisation des trouées dans des conditions où la reproduction sexuée serait limitée par la destruction des structures reproductrices (Fahrig *et al*. 1994, Klimešová & Klimeš 2003). A l'inverse, nous n'avons pas montré de relation entre la résistance au pâturage et le nombre et la biomasse de fleurs produites (ARTICLE 3). Chez les formes clonales décrites en prairies pâturées et en réponse à la défoliation, la dissémination par les graines émerge comme le seul moyen de coloniser l'espace. En conditions pâturées, celle-ci est rendue d'autant plus efficace que les herbivores peuvent constituer des vecteurs de propagules (endo- ou épizoochorie, Amiaud *et al*. 2000, Moussie *et al.* 2005, Couvreur *et al.* 2008). La diminution de la pression compétitive, notamment pour la lumière, générée par la défoliation des plantes voisines facilite, quant à elle, l'émergence des graines et l'établissement des jeunes plantules. L'avantage de la clonalité en prairies pâturées reposerait donc essentiellement sur les capacités de stockage qu'elle confère au fragment clonal. D'une part, en permettant une repousse rapide et efficace des tissus endommagés, les stocks de réserves contenus dans la base des tiges confèreraient un avantage compétitif crucial en conditions pâturées. D'autre part, en permettant le développement de méristèmes floraux activés suite à la défoliation, ils favoriseraient la reproduction sexuée et la dissémination.

A l'issue de ce travail de thèse, il apparaît donc particulièrement intéressant de développer des études permettant d'estimer l'importance de la reproduction sexuée sous divers régimes de pâturage, et notamment de vérifier l'impact du pâturage sur les compromis entre reproduction sexuée et multiplication clonale.

REFERENCES BIBLIOGRAPHIQUES

Adler PB, Milchunas DG, Sala OE, Burke IC & Lauenroth WK (2005) Plant traits and ecosystem grazing effects: comparison of U.S. sagebrush steppe and Patagonian Steppe. *Ecological Applications*, 15: 774-792.

Adler PB, Raff DA & Laurenroth WK (2001) The effect of grazing on the spatial heterogeneity of vegetation. *Oecologia*, 128: 465-479.

Agrawal AA (2001) Tansgenerational consequences of plant responses to herbivory: an adaptive maternal effect? *American Naturalist*, 157: 555-569.

Alados CL, Aich AE, Komac B, Pueyo Y & García-Gonzalez (2007) Self-organized spatial patterns of vegetation in alpine grasslands. *Ecological Modelling*, 201: 233-242.

Albrecht G, Biemelt S & Baumgartner S (1997) Accumulation of fructans following oxygen deficiency stress in related plant species with different flooding tolerances. *New Phytologist*, 136: 137-144.

Albrecht G, Kammerer S, Praznik W & Wiedenroth EM (1993) Fructan content of wheat seedlings (*Triticum aestivum* L.) under hypoxia and following re-aeration. *New Phytologist*, 1123: 471-476.

Alpert P (1991) Nitrogen sharing among ramets increases clonal growth in *Fragaria Chiloensis*. *Ecology*, 72: 69-80.

Alpert P (1996) Nutrient sharing in natural clonal fragments of *Fragaria chiloensis*. *Journal of Ecology*, 84: 395-406.

Alpert P & Simms EL (2002) The relative advantages of plasticity and fixity in different environments: when is it good for a plant to adjust? *Evolutionary Ecology*, 16: 285-297.

Alpert P & Stuefer JF (1997) Division of labour in clonal plants. In: de Kroon H & van Groenendael J (eds) *The ecology and evolution of clonal plants*. Backhuys Publishers, Leiden, pp. 137-154.

Amiard V, Morvan-Bertrand A, Billard JP, Huault C, Keller F & Prud'homme MP (2003a) Fructans, but not the sucrosyl-galactosides, raffinose and loliose, are affected by drought stress in perennial ryegrass. *Plant Physiology*, 132: 2218-2229.

Amiard V, Morvan-Bertrand A, Billard JP, Huault C & Prud'homme MP (2003b) Fate of fructose supplied to leaf sheaths after defoliation of *Lolium perenne* L.: assessment by ^{13}C-fructose labeling. *Journal of Experimental Botany*, 54: 1231-1243.

Amiaud B (1998) *Dynamique végétale d'un écosystème prairial soumis à différentes modalités de pâturage. Exemple des communaux du Marais Poitevin*. Thèse de 3ème cycle, Université de Rennes 1.

Amiaud B, Bonis A & Bouzillé JB (2000) Conditions de germination et rôle des herbivores dans la dispersion et le recrutement d'une espèce clonale : *Juncus gerardi* Lois. *Canadian Journal of Botany*, 78 : 1430-1439.

Anderson TM, McNaughton SJ & Ritchie ME (2004) Scale-dependent relationships between the spatial distribution of a limiting resource and plant species diversity in an African grassland ecosystem. *Oecologia*, 139: 277-287.

Anderson VJ & Briske DD (1995) Herbivore-induced species replacement in grasslands: is it driven by herbivory tolerance or avoidance? *Ecological Applications*, 5: 1014-1024.

Archer S & Delting JK (1984) The effect of defoliation and competition on regrowth of tillers of two North American mixed-grass prairie graminoids. *Oikos*, 43: 351-357.

Asaeda T, Rajapakse L, Manatunge J & Sahara N (2006) The effect of summer harvesting of *Phragmites australis* on growrth characteristics and rhizome resource storage. *Hydrobiologia*, 553: 327-335.

Augustine DJ (2003) Spatial heterogeneity in the herbaceous layer of a semi-arid savanna ecosystem. *Plant Ecology*, 167: 319-332.

Augustine DJ & Frank DA (2001) Effects of migratory grazers on spatial heterogeneity of soil nitrogen properties in a grassland ecosystem. *Ecology*, 82: 3149-3162.

Bakker C, Blair JM & Knapp AK (2003) Does resource availability, resource heterogeneity or species turnover mediate changes in plant species richness in grazed grasslands? *Oecologia*, 137, 385-391.

Bakker ES, Ritchie ME, Olff H, Milchunas DG & Knops JMH (2006) Herbivore impact on grassland diversity depends on habitat productivity and herbivore size. *Ecology Letters*, 9: 780-788.

Bakker JP, de Leeuw J & van Wieren E (1983) Micro-patterns in grassland vegetation created and sustained by sheep-grazing. *Vegetatio*, 55: 153-161.

Banach K, Banach AM, Lamers LPM, de Kroon H, Bennicelli RP, Smits AJM & Visser EJW (2009) Differences in flooding tolerance between species from two wetland habitats with contrasting hydrology: implications for vegetation development in future floodwater retention areas. *Annals of Botany*, 103: 341-351.

Barney JN, DiTommaso A, Weston LA (2005) Differences in invisibility of two contrasting habitats and invasiveness of two mugwort *Artemisia vulgaris* populations. *Journal of Applied Ecology*, 42: 567-576.

Barney JN, Whitlow TH & DiTommaso A (2009) Evolution of an invasive phenotype: shift to belowground dominance and enhances competitive ability in the introduced range. *Plant Ecology*, 202: 275-284.

Barrat-Segretain MH, Bornette G & Hering-Vilas-Bôas A (1998) Comparative abilities of vegetative regeneration among aquatic plants growing in disturbed habitats. *Aquatic Botany*, 60: 201-211.

Beaulieu J, Gauthier G & Rochefort L (1997) The growth response of graminoid plants to goose grazing in a High Arctic environment. *Journal of Ecology*, 84: 905-914.

Bell AD (1979) The hexagonal branching pattern of rhizomes of *Alpinia speciosa* L. (Zingiberaceae). *Annals of Botany*, 43: 09-223.

Bell AD (1984) Dynamic morphology: a contribution to plant population ecology. In: Dirzo R & Sarukhán J (eds) *Perspectives in Plant Population Ecology*. Sinauer, Sunderland, Massachusetts, pp 48-65.

Bell AD & Tomlinson PB (1980) Adaptive architecture in rhizomatous plants. *Botanical Journal of the Linnean Society*, 80: 125-160.

Bell TL & Ojeda F (1999) Underground starch storage in *Erica* species of the Cape Floristic Region – differences between seeders and resprouters. *New Phytologist*, 144: 143-152.

Bellingham PJ & Sparrow AD (2000) Resprouting as a life history strategy in woody plant communities. *Oikos*, 89: 409-416.

Belsky AJ (1987) The effects of grazing: confounding of ecosystem, community, and organism scale. *American Naturalist*, 129: 777-783.

Birch CPD & Hutchings MJ (1994) Exploitation of patchily distributed soil resources by the clonal herb *Glechoma hederacea*. *Journal of Ecology*, 82: 653-664.

Blom CWPM (1999) Adaptations to flooding stress: from plant community to molecule. *Plant Biology*, 1: 261-273.

Blom CWPM & Voesenek LACJ (1996) Flooding: the survival strategies of plants. *Trends in Ecology and Evolution*, 11: 290-295.

Bond WJ & Midgley JJ (2001) Ecology of sprouting in woody plants: the persistence niche. *Trends in Ecology and Evolution*, 16: 45-51.

Bond WJ & Midgley JJ (2003) The evolutionary ecology of sprouting in woody plants. *International Journal of Plant Science*, 164(Suppl.): S103-S114.

Bonis A, Bouzillé JB, Amiaud B & Loucougaray G (2005) Plant community patterns in old embanked grasslands and the survival of halophytic flora. *Flora*, 200: 74-87.

Bouzillé JB (1992) *Structure et dynamique des paysages, des communautés et des populations végétales des marais de l'ouest*. Thèse d'état, spécialité écologie. Université de Rennes 1.

Bouzillé JB & Tournade F (1990) *Sols et végétation des prairies naturelles humides du Marais Poitevin. L'exemple des marais communaux.* Rapport d'étude, Parc Naturel Régional du Marais Poitevin ; Ministère de l'Agriculture.

Bradshaw AD (1965) Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics*, 13: 115-155.

Bråthen KA, Agrell J, Berteaux D & Jónsdóttir IS (2004) Intraclonal variation in defence substances and palatability: a study on *Carex* and lemmings. *Oikos*, 105: 461-470.

Bråthen KA & Junttila O (2006) Infertile times: response to damage in genets of the clonal sedge *Carex bigelowii*. *Plant Ecology*, 187: 83-95.

Briske DD (1996) Strategies of plant survival in grazed systems: a functional interpretation. In: Hodgson J & Illius AW (eds) *The Ecology and management of grazing systems*. CAB International, Wallingford, pp 33-67.

Briske DD & Anderson VJ (1992) Competitive ability of the bunchgrass *Schizchyrium scoparium* as affected by grazing history and defoliation. *Vegetatio*, 103: 41-49.

Briske DD & Silvertown JW (1993) Plant demography and grassland community balance: the contribution of population regulation mechanisms. In: Baker MJ (ed) *Proceedings of the XVII International Grassland Congress*. SIR Publishing, Wellington, pp 291-298.

Brocklebank KJ & Hendry JAF (1989) Characteristics of plant species which store different types of reserve carbohydrates. *New Phytologist*, 112: 255-263.

Brown B & Allen T (1989) The importance of scale in evaluating herbivory impacts. *Oikos*, 54: 189-194.

Brown JR & Stuth JW (1993) How herbivory affects grazing tolerant and sensitive grasses in a central Texas grassland: integrating plant response across hierarchical levels. *Oikos*, 67: 291-298.

Brun FG, Cummaudo F, Olivé I, Vergara JJ & Pérez-Lloréns JL (2007) Clonal extent, apical dominance and network in features in the phalanx angiosperm *Zostera noltii* Hornem. *Marine Biology*, 151: 1917-1927.Belsky AJ (1986) Does herbivory benefit plants? A review of the evidence. *American Naturalist*, 127: 870-892.

Bullock JM, Clear-Hill B & Silvertown J (1994) Tiller dynamics of two grasses - responses to grazing, density and weather. *Journal of Ecology*, 82: 331-340.

Bullock JM, Franklin J, Stevenson MJ, Silvertown J, Coulson SJ, Gregory SJ & Softs R (2001) A plant trait analysis of responses to grazing in a long-term experiment. *Journal of Applied Ecology*, 38: 253-267.

Cain ML (1994) Consequences of foraging in clonal plant species. *Ecology*, 75: 933-944.

Cain ML & Damman H (1997) Clonal growth and ramet performance in the woodland herb, *Asarum canadense*. *Journal of Ecology*, 85: 883-897

Cairns AJ, Cookson A, Thomas BJ & Turner LB (2002) Starch metabolism in the fructan-grasses: patterns of starch accumulation in excised leaves of *Lolium temulentum* L. *Journal of Plant Physiology*, 159: 293-305.

Callaghan TV, Svensson BM, Bowmann H, Lindley DK & Carlsson BA (1990) Models of clonal plant growth based on population dynamics and architecture. *Oikos*, 75: 257-269.

Caraco T & Kelly CK (1990) On the adaptive value of physiological integration in clonal plants. *Ecology*, 72: 81-93.

Chaneton EJ & Facelli JM (1991) Disturbance effects on plant community diversity: spatial scales and dominance hierarchies. *Plant Ecology*, 93: 143-155.

Chapin FS III (1980) The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics*, 11: 233-260.

Chapin FS III, Schulze ED & Mooney HA (1990) The ecology and economics of storage in plants. *Annual Review of Ecology and Systematics*, 21: 423-447.

Charpentier A, Mesléard F & Thompson JD (1998) The effect of rhizome severing on the clonal growth and clonal architecture of *Scirpus maritimus*. *Oikos*, 83: 107-116.

Charpentier A & Stuefer JF (1999) Functional specialization of ramets in *Scirpus maritimus*. *Plant Ecology*, 141: 129-136.

Chatterton NJ, Harrisson PA, Bennett JH, Asay KH (1989) Carbohydrate portioning in 185 accessions of Gramineae grown under warm and cool temperatures. *Journal of Plant Physiology*. 134: 169-179.

Cheplick GP & Chui T (2001) Effect of competitive stress on vegetative growth, storage, and regrowth after defoliation in *Phleum pratense*. *Oikos*, 95: 291-299.

Cheplick GP & Gutierrez CM (2000) Clonal growth and storage in relation to competition in genets of the rhizomatous perennial *Amphibromus scabrivalvis*. *Canadian Journal of Botany*, 78: 537-546.

Chessel D, Dufour AB & Thioulouse J (2004) The ade4 package – I – One-table methods. *R News*, 4: 5-10.

Cliff AD & Ord JK (1973) *Spatial autocorrelation.* Pion, London.

Cliff AD & Ord JK (1981) *Spatial processes: models and applications.* Pion, London.

Cole DN (1995) Experimental trampling of vegetation. II. Predictors of resistance and resilience. *Journal of Applied Ecology*, 32: 215-224.

Collins SL, Knapp AK, Briggs JM, Blair JM & Steinauer EM (1998) Modulation of diversity by grazing and mowing in native tallgrass prairie. Science, 280: 745-747.

Collins SL & Smith MD (2006) Scale-dependent interaction of fire and grazing on community heterogeneity in tallgrass prairie. *Ecology*, 87: 2058-2067.

Couvreur M, Verheyen K, Vellend M, Lamoot I, Cosyns E, Hoffmann M & Hermy M (2008) Epizoochory by large herbivores: merging data with models. *Basic and Applied Ecology*, 9: 204-212.

Crawford RMM (1996) Whole plant adaptations to fluctuating water tables. *Folia Geobotanica and Phytotaxonomica*, 31: 7-24.

Crawford RMM & Brändle R (1996) Oxygen deprivation stress in a changing environment. *Journal of Experimental Botany*, 47: 145-159.

Crawley MJ (2007) *The R book*. John Wiley & Sons, Chichester.

D'Hertefeld T & Falkengren-Grerup U (2002) Extensive physiological integration in *Carex arenaria* and *Carex disticha* in relation to potassium and water availability. *New Phytologist*, 156: 469-477.

D'Hertefeldt T & Jónsdóttir IS (1999) Extensive physiological integration in intact clonal systems of *Carex arenaria*. *Journal of Ecology*, 87: 258-264.

de Bello F, Lepš J & Sebastià MT (2005) Predictive value of plant traits to grazing along a climatic gradient in the Mediterranean. *Journal of Applied Ecology* 42: 824-833.

de Bello F, Lepš J & Sebastià MT (2006) Variations in species and functional plant diversity along climatic and grazing gradients. *Ecography*, 29: 801-810.

de Jong T & van der Meijden E (2000) On the correlation between allocation to defence and regrowth in plants. *Oikos*, 88: 503-508.

de Kroon H, Huber H, Stuefer JF & van Groenendael JM (2005) A modular concept of phenotypic plasticity in plants. *New Phytologist*, 166: 73-82.

de Kroon H & Hutchings MJ (1995) Morphological plasticity in clonal plants: the foraging concept reconsidered. Journal of Ecology, 83: 143-152.

de Kroon H & Knops J (1990) Habitat exploration through morphological plasticity in two chalk grassland perennials. *Oikos*, 59: 39-49.

de Kroon H & Kwant R (1991) Density-dependent growth responses in two clonal herbs: regulation of shoot density. *Oecologia*, 86: 298-304.

de Kroon H & Schieving F (1990) Resource partitioning in relation to clonal growth strategy. In: van Groenendael J & de Kroon H (eds) *Clonal growth in plants: regulation and function*. SPB Academic Publishing, The Hague, pp 113-130.

de Visser R, Vianden H & Schnyder H (1997) Kinetics and relative significance of remobilized and current C and N incorporation in leaf and root growth zones of *Lolium perenne* after defoliation: assessment by ^{13}C and ^{15}N steady-state labelling. *Plant, Cell and Environment*, 20: 37-46.

Del Tredici P (2001) Sprouting in temperate trees: a morphological and ecological review. *Botanical Review*, 67: 121-140.

del-Val E & Crawley MJ (2004) Importance of tolerance to herbivory for plant survival in a British grassland. *Journal of Vegetation Science*, 15: 357-364.

del-Val E & Crawley MJ (2005) Are grazing increaser species better tolerators than decreasers? An experimental assessment of defoliation tolerance in eight British grassland species. *Journal of Ecology*, 93: 1005-1016.

Derner JD & Briske DD (2001) Below-ground carbon and nitrogen accumulation in perennial grasses: a comparison of caespitose and rhizomatous growth forms. *Plant and Soil*, 237: 117-127.

Diaz S, Cabido M & Casanoves F (1998) Plant functional traits and environmental filters at a regional scale. *Journal of Vegetation Science*, 9: 113-122.

Diaz S, Lavorel S, McIntyre S, Falczuk V, Casanoves F, Milchunas DG, Skarpe C, Rusch G, Sternberg M, Noy-Meir I, Landsberg J, Zhang W, Clark H & Campbell BD (2007) Plant trait response to grazing – a global synthesis. *Global Change Biology*, 13: 313-341.

Diaz S, Noy-Meir I & Cabido M (2001) Can grazing response of herbaceous plants be predicted from simple vegetative traits? *Journal of Applied Ecology*, 38: 497-508.

Dolédec S & Chessel D (1991) Recent development in linear ordination methods for environmental sciences. *Advances in Ecology*, 1: 133-155.

Dong M (1993) Morphological plasticity of the clonal herb *Lamiastrum galeobdolon* (L.) Ehrend. & Polatschek in response to partial shading. *New Phytologist*, 124: 291-300.

Dong M & de Kroon H (1994) Plasticity in morphology and biomass allocation in *Cynodon dactylon*, a grass species forming stolons and rhizomes. *Oikos*, 70: 99-106.

Dong M & Pierdominici MG (1995) Morphology and growth of stolons and rhizomes in three clonal grasses, as affected by different light supply. *Vegetatio*, 116: 25-32.

Dykserhuis EJ (1949) Condition and management of rangelands based on quantitative ecology. *Journal of Range Management*, 2: 104-115.

Eriksson O (1986) Mobility and space capture in the stoloniferous plant *Potentilla anserina*. *Oikos*, 46: 82-87.

Eriksson O (1989) Seedling dynamics and life histories in clonal plants. *Oikos*, 55: 231-238.

Eriksson O (1992) Evolution of seed dispersal and recruitment in clonal plants. *Oikos*, 63: 439-448.

Eriksson O (1997) Clonal life histories and the evolution of seed recruitment. In: de Kroon H & van Groenendael J (eds) *The ecology and evolution of clonal plants*. Backhuys Publishers, Leiden, pp. 211-226.

Eriksson O & Jerling L (1990) Hierarchical selection and risk spreading in clonal plants. In: van Groenendael J & de Kroon H (eds) *Clonal growth in plants: regulation and function*. SPB Academic Publishing, The Hague, pp 79-94.

Evette A, Bédécarrats A & Bornette G (2009) Environmental constraints influence clonal traits of herbaceous plant communities in an Alpine Massif. Folia Geobotanica, 44: 95-108.

Fahrig L, Coffin DP, Lauenroth WK & Shugart HH (1994) The advantage of long-distance spreading in highly disturbed habitats. *Evolutionary Ecology*, 8: 172-187.

Ferraro DO & Oesterheld M (2002) Effect of defoliation on grass growth. A quantitative review. *Oikos*, 98: 125-133.

Fischer M & van Kleunen M (2002) On the evolution of clonal plant life histories. Evolutionay *Ecology*, 15: 565-582.

Fitter AH (1987) An architectural approach to the comparative ecology of plant root systems. *New Phytologist*, 105(Suppl): 61-77.

Fitter AH, Stickland TR, Harvey DM & Wilson GW (1991) Architectural analysis of plant root systems. 1. Architectural correlates of exploitation efficiency. *New Phytologist*, 118: 375-382.

Fuhlendorf SD & Smeins FE (1999) Scaling effects of grazing in a semi-arid grassland. *Journal of Vegetation Science*, 10: 731-738.

García MB & Ehrlén J (2002) Reproductive effort and herbivory timing in a perennial herb: fitness components at the individual and population levels. *American Journal of Botany*, 89, 1295-1302.

Gardner SN & Mangel M (1997) When can a clonal organism escape senescence? *American Naturalist*, 150: 462-490.

Getz WM & Wilmers CC (2004) A local nearest-neighbor convex-hull construction of home ranges and utilization distributions. *Ecography*, 27: 489-505.

Gold WG & Caldwell MM (1989) The effects of the spatial pattern of defoliation on regrowth of a tussock grass. I. Growth responses. *Oecologia*, 80: 289-296.

Goldberg DE & Landa K (1991) Competitive effect and response: hierarchies and correlated traits in the early stages of competition. *Journal of Ecology*, 79: 1013-1030.

Gómez S, Latzel V, Verhulst YM & Stuefer JF (2007) Costs and benefits of induced resistance in a clonal plant network. *Oecologia*, 153: 921-930.

Gómez S, Onoda Y, Ossipov V & Stuefer JF (2008) Systemic induced resistance: a risk-spreading strategy in clonal plant networks? *New Phytologist*, 179: 1142-1153.

Gómez S & Stuefer JF (2006) Members only: induced systemic resistance to herbivory in a clonal plant network. *Oecologia*, 147: 461-468.

Grace J (1993) The adaptive significance of clonal reproduction in angiosperms: an aquatic perspective. *Aquatic Botany*, 44: 159-180.

Grime JP (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist*, 111: 1169-1194.

Gross N, Suding K & Lavorel S (2007) Leaf dry matter content and lateral spread predict response to land use change for six subalpine grassland species. *Journal of Vegetation Science*, 18: 289-300.

Grubb P (1977) The maintenance of species richness in plant communities: the importance of the regeneration niche. *Biological Review*, 52: 107-145.

Halassy M, Campetella G, Canullo R & Mucina L (2005) Patterns of functional clonal traits and clonal growth modes in contrasting grasslands in the central Apennines, Italy. *Journal of Vegetation Science*, 16: 29-36.

Harper JL (1977) *Population Biology of Plants*. Academic Press, London.

Harper JL & White J (1974) The demography of plants. *Annual Review of Ecology and Systematics*, 5: 419-463.

Hartnett DC (1989) Density- and growth stage-dependent responses to defoliation in two rhizomatous grasses. *Oecologia*, 80: 414-420.

Hartnett DC & Bazzaz FA (1983) Physiological integration among intraclonal ramets in *Solidago Canadensis* L. *Ecology*, 64: 779-788.

Haukioja E & Koricheva J (2000) Tolerance in woody *vs.* herbaceous plants. *Evolutionary Ecology*, 14: 551-562.

Hawker JS (1985) Sucrose. In: Dey PM & Dixon RA (eds) *Biochemistry of storage carbohydrates in green plants*. Academic Press, London, pp 1-51.

Hay MJM & Newton PCD (1996) Effect of severity of defoliation on the viability of reproductive and vegetative axillary buds of *Trifolium repens* L. *Annals of Botany*, 78: 117-123.

Hendry G (1987) The ecological significance of fructan in a contemporary flora. *New Phytologist*, 106: 201-216.

Henry GM, Burton MG & Yelverton FH (2007) Effect of mowing on lateral spread and rhizome growth of troublesome *Paspalum* species. *Weed Science*, 55: 486-490.

Herben T, During HJ & Law R (2000) Spatio-temporal patterns in grassland communities. In: Dieckmann U, Law R & Metz JHJ (eds) *The geometry of ecological interactions: simplifying spatial complexity*. Cambridge University Press, New York, pp. 48–64.

Herben T & Hara T (1997) Competition and spatial dynamics of clonal plants. In: de Kroon H & van Groenendael J (eds) *The ecology and evolution of clonal plants*. Backhuys Publishers, Leiden, pp 331-358.

Herben T & Hara T (2003) Spatial pattern formation in plant communities. In: Sekimura T, Noji S, Ueno N & Maini PK (eds) *Morphogenesis and pattern formation in biological systems–experiments and models*. Springer Verlag, Berlin, pp 223–235.

Herben T, Hara T, Marshall C & Soukupova L (1994) Plant clonality: biology and diversity. *Folia Geobotanica and Phytotaxonomica*, 29: 113-122.

Herben T & Suzuki JI (2002) A simulation study of the effects of architectural constraints and resource translocation on population structure and competition in clonal plants. *Evolutionary Ecology*, 15: 403-423.

Herman EM & Larkins BA (1998) Protein storage bodies and vacuoles. *The Plant Cell*, 11: 601-613.

Herms DA & Mattson WJ (1992) The dilemma of plants: to grow or defend. *Quarterly Review of Biology*, 67: 283–335.

Hicks S & Turkington R (2000) Compensatory growth of three herbaceous perennial species: the effect of clipping and nutrient availability. *Canadian Journal of Botany*, 78: 759-767.

Huber H & During HJ (2001) No long-term costs of meristem allocation to flowering in stoloniferous *Trifolium* species. *Evolutionary Ecology*, 14: 731-748.

Huber H & Hutchings MJ (1997) Differential response to shading in orthotropic and plagiotropic shoots of the clonal herb *Glechoma hirsuta*. *Oecologia*, 112: 485-491.

Huber H, Lukacs S & Watson MA (1999) Spatial structure of stoloniferous herbs: an interplay between structural blue-print, ontogeny and phenotypic plasticity. *Plant Ecology*, 141: 107-115.

Huber H & Stuefer JF (1997) Shade-induced changes in the branching pattern of a stoloniferous herb: functional response or allometric effect? *Oecologia*, 110: 478-486.

Hulme PE (1996) Herbivory, plant regeneration, and species coexistence. *Journal of Ecology*, 84, 609-615.

Huntly N (1991) Herbivores and the dynamic of communities and ecosystems. *Annual Review of Ecology and Systematics*, 22: 477-503.

Hutchings MJ (1999) Clonal plants as cooperative systems: benefits in heterogeneous environments. *Plant Species Biology*, 14: 1-10.

Hutchings MJ & Bradbury IK (1986) Ecological perspectives on clonal perennial herbs. *BioScience* 36: 178-182.

Hutchings MJ & de Kroon H (1994) Foraging in plants: the role of morphological plasticity in resource acquisition. *Advances in Ecological Research*, 25: 159-238.

Hutchings MJ & Mogie M (1990) The spatial structure of clonal plants: control and consequences. In: van Groenendael J & de Kroon H (eds) *Clonal growth in plants: regulation and function*. SPB Academic Publishing, The Hague, pp 57-78.

Hutchings MJ & Wijesinghe D (1997) Patchy habitats, division of labour and growth dividends in clonal plants. *Trends in Ecology and Evolution*, 12: 390-394.

Ikegami M, Whigham DF & Werger MJA (2007) Responses of rhizome length and ramet production to resource availability in the clonal sedge *Scirpus olneyi* A. Gray. *Plant Ecology*, 189: 247-259.

Insausti P, Chaneton EJ & Soriano A (1999) Flooding reverted grazing effects on plant community structure in mesocosms of lowland grassland. *Oikos*, 84: 266-276.

Iwasa Y & Kubo T (1997) Optimal size of storage for recovery after unpredictable disturbances. *Evolutionary Ecology*, 11: 41-65.

Jackson MB & Drew MC (1984) Effect of flooding on herbaceous plants. In: Kozlowski TT (ed) *Flooding and plant growth.* Academic Press, London, pp 47-128.

Jaeger CH & Monson RK (1992) Adaptive significance of nitrogen storage in *Bistorta bistortoides*, an alpine herb. *Oecologia*, 92: 578-585.

Jarvis SC & Macduff JH (1989) Nitrate nutrition of grasses from steady-state supplies in flowing solution culture following nitrate deprivation and/or defoliation. I. Recovery of uptake and growth and their interactions. *Journal of Experimental Botany*, 218: 965-975.

Jiaseński M & Bazzaz FA (1999) The fallacy of ratios and the testability of models in biology. *Oikos*, 84: 321-326.

John P (1992) *Biosynthesis of the major crop products.* John Wiley & Sons, Chichester.

Jónsdóttir IS (1991) Effects of grazing on tiller size and population dynamics in a clonal sedge (*Carex bigelowii*). *Oikos*, 62: 177-188.

Jónsdóttir IS, Augner M, Fagerström T, Persson H & Stenström (2000) Genet age in marginal populations of two clonal *Carex* species in the Siberian Arctic. *Ecography*, 23: 402-412.

Jónsdóttir IS & Callaghan TV (1988) Interrelationships between different generations of interconnected tillers of Carex bigelowii. *Oikos*, 52: 120-128.

Jónsdóttir IS & Callaghan TV (1989) Localized defoliation stress and the movement of ^{14}C-photoassimilates between tillers of *Carex bigelowii*. *Oikos*, 54: 211-219.

Jónsdóttir IS & Callaghan TV (1990) Intraclonal translocation of ammonium and nitrate nitrogen in *Carex bigelowii* Torr. ex Schwein. using ^{15}N and nitrate reductase assays. *New Phytologist*, 114: 419-428.

Jónsdóttir IS & Watson MA (1997) Extensive physiological integration: an adaptive trait in resource-poor environments? In: de Kroon H & van Groenendael J (eds) *The ecology and evolution of clonal plants*. Backhuys Publishers, Leiden, pp 109-136.

Juenger T & Lennartson T (2000) Tolerance in plant ecology and evolution: toward a more unified theory of plant-herbivore interaction (Preface). *Evolutionary Ecology*, 14: 283-287.

Jutila H (1999) Effect of grazing on the vegetation of shore meadows along the Bothnian Sea, Finland. *Plant Ecology*, 140: 77–88.

Kavanova M & Gloser V (2005) The use of internal nitrogen stores in the rhizomatous grass *Calamagrostis epigejos* during regrowth after defoliation. *Annals of Botany*, 95: 457-463.

Keddy PA (1992) Assembly and response rules: two goals for predictive community ecology. *Journal of Vegetation Science*, 3: 157-164.

Kelly CK (1995) Thoughts on clonal integration: facing the evolutionary context. *Evolutionary Ecology*, 9: 575-585.

Kleijn D, Treier UA & Müller-Schärer H (2005) The importance of nitrogen and carbohydrate storage for plant growth of the alpine herb *Veratum album*. *New Phytologist*, 166: 565-575.

Kleijn D & van Groenendael J (1999) The exploitation of heterogeneity by a clonal plant in habitats with contrasting productivity levels. *Journal of Ecology*, 87: 873-884.

Kleyer M, Bekker RM, Knevel IC, Bakker JP, Thompson K, Sonnenschein M, Poschlod P, van Groenendael JM, Klimeš L, Klimešová J, Klotz S, Rusch GM, Hermy M, Adriaens D, Boedeltje G, Bossuyt B, Dannemann A, Endels P, Götzenberger L, Hodgson JG, Jackel AK, Kühn I, Kunzmann D, Ozinga WA, Römermann C, Stadler M, Schlegelmilch J, Steendam HJ, Tackenberg O, Wilmann B, Cornelissen JHC, Eriksson O, Garnier E & Peco B (2008) The LEDA Traitbase: A database of life-history traits of Northwest European flora. *Journal of Ecology*, 96: 1266-1274.

Klimeš L & Klimešovà J (1999) CLO-PLA2 – A database of clonal plants in central Europe. *Plant Ecology*, 141: 9-19.

Klimeš L & Klimešová J (2002) The effects of mowing and fertilization on carbohydrate reserves and regrowth of grasses: do they promote plant coexistence in species-rich meadows? *Evolutionary Ecology*, 15: 363-382.

Klimeš L, Klimešová J & Čižková H (1999) Carbohydrate storage in rhizomes of *Phragmites australis*: the effect of altitude and rhizome age. *Aquatic Botany*, 64: 105-110.

Klimeš L, Klimešovà J, Hendricks R & van Groenendael J (1997) Clonal plant architecture: a comparative analysis of form and function. In: de Kroon H & van Groenendael J (eds) *The ecology and evolution of clonal plants*. Backhuys Publishers, Leiden, pp 1-29.

Klimešovà J & de Bello F (2009) CLO-PLA: the database of clonal and bud bank traits of Central European flora. *Journal of Vegetation Science*, 20: 511-516.

Klimešová J & Klimeš L (2003) Resprouting of herbs in disturbed habitats: is it adequately described by Bellingham - Sparrow's model? *Oikos*, 103: 225-229.

Klimešovà J & Klimeš L (2007) Bud banks and their role in vegetative regeneration – A literature review and proposal for simple classification assessement. *Perspectives in Plant Ecology, Evolution and Systematics*, 8: 115-129.

Klimešovà J & Klimeš L (2008) Clonal growth diversity and bud banks of plants in the Czech flora: an evaluation using the CLO-PLA3 database. *Preslia*, 80: 255-275.

Klimešovà J, Latzel V, de Bello F & van Groenendael JM (2008) Plant functional traits in studies of vegetation changes in response to grazing and mowing: towards a use of more specific traits. *Preslia*, 80: 245-253.

Knox KJE & Clarke PJ (2005) Nutrient availability induces contrasting allocation and starch formation in resprouting and obligate seedling shrubs. *Functional Ecology*, 19: 690-698.

Kobe RK (1997) Carbohydrate allocation to storage as a basis of interspecific variation in sapling survivorship and growth. *Oikos*, 226-233.

Kohler F, Gillet F, Gobat JM & Buttler A (2004) Seasonal vegetation changes in mountain pastures due to simulated effects of cattle grazing. *Journal of Vegetation Science*, 15: 143-150.

Kohyani PT, Bossuyt B, Bonte D & Hoffmann M (2008) Grazing as a management tool in dune grasslands : evidence of soil and scale dependence of the effect of large herbivores on plant diversity. *Ecological Conservation*, 141: 1687-1694.

Koubek T & Herben T (2008) Effect of systemic diseases on clonal integration: modeling approach. *Evolutionary Ecology*, 22: 449-460.

Kun A & Oborny B (2003) Survival and competition of clonal plant populations in spatially and temporally heterogeneous habitats. *Community Ecology*, 4: 1-20.

Landa K, Benner B, Watson MA & Gartner J (1992) Physiological integration for carbon in mayapple (*Podophyllum peltatum*), a clonal perennial herb. *Oikos*, 63: 348-356.

Lasso E, Engelbrecht BMJ, Dalling JW (2009) When sex is not enough: ecological correlates of resprouting capacity in congeneric tropical forest shrubs. *Oecologia*, 161: 43-56.

Lattanzi FA, Schnyder H & Thornton B (2004) Defoliation effects on carbon and nitrogen substrate import and tissue-bound efflux in leaf growth zones of grasses. *Plant, Cell and Environment*, 27: 347-356.

Latzel V, Mihulka S & Klimešovà J (2008) Plant traits and regeneration of urban plant communities after disturbance: does the bud bank play any role? *Applied Vegetation Science*, 11: 387-394.

Lavorel S & Garnier E (2002) Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional Ecology*, 16: 545-556.

Lavorel S, McIntyre S. Landsberg J & Forbes TDA (1997) Plant functional classifications: from general groups to specific groups based on response to disturbance. *Trends in Ecology and Evolution*, 12: 474-478.

Legendre P & Legendre L (1998). *Numerical Ecology*. 2nd English ed. Elsevier, Amsterdam.

Lennartsson T, Nilsson P & Tuomi J (1998) Induction of overcompensation in the field gentian, *Gentianella campestris*. *Ecology*, 79: 1061-1072.

Lenssen JPM, van Kleunen M, Fischer M & de Kroon H (2004). Local adaptation of clonal plant *Ranunculus reptans* to flooding along a small-scale gradient. *Journal of Ecology*, 92: 696-706.

Li B, Shibuya T, Yogo Y & Hara T (2004) Effect of ramet clipping and nutrient availability on growth and biomass allocation of yellow nutsedge. *Ecological Research*, 19: 603-612.

Liao M, Yu F, Song M, Zhang S, Zhang J & Dong M (2003) Plasticity in R/S ratio, morphology and fitness-related traits in response to reciprocal patchiness of light and nutrients in the stoloniferous herb, *Glechoma longituba* L. *Acta Oecologica*, 24: 231-239.

Lipson DA, Bowman WD & Monson RK (1996) Luxury uptake and storage of nitrogen in the rhizomatous alpine herb, *Bistorta bistortoides*. *Ecology*, 77: 1277-1285.

López F, Serrano JM & Acosta FJ (1994) Parallels between the foraging strategies of ants and plants. *Trends in Ecology and Evolution*, 9: 150-153.

Louahlia S, Lainé P, Thornton B & Boucaud J (2000) The role of N-remobilisation and the uptake of NH_4^+ and NO_3^- by *Lolium perenne* L. in laminae growth following defoliation under field conditions. *Plant and Soil*, 220: 175-187.

Louahlia S, Macduff JH, Ourry A, Humphreys M & Boucaud J (1999) Nitrogen reserve status affects the dynamics of nitrogen remobilization and mineral nitrogen uptake during recovery of contrasting cultivars of *Lolium perenne* from defoliation. *New Phytologist*, 142: 451-462.

Loucougaray G (2003) *Régimes de pâturage et hétérogénéité de la structure et du fonctionnement de la végétation prairiale (Marais Poitevin).* Thèse de 3ème cycle, Université de Rennes 1.

Loucougaray G, Bonis A & Bouzillé JB (2004) Effects of grazing by horses and/or cattle on the diversity of coastal grasslands in western France. *Biological Conservation*, 116: 59-71.

Lovett-Doust L (1981) Population dynamics and local specialization in a clonal perennial (*Ranunculus repens*). I. The dynamics of ramets in contrasting habitats. *Journal of Ecology*, 69:743-755.

MacDonald & Lieffers 1993 MacDonald SE, Lieffers VJ (1993) Rhizome plasticity and clonal foraging of *Calamagrostis canadensis* in response to habitat heterogeneity. *Journal of Ecology*, 81: 769-776.

Macek P & Lepš J (2003) The effect of environmental heterogeneity on clonal behaviour of *Prunella vulgaris* L. *Plant Ecology*, 168: 31-43.

Mack RN & Thompson JN (1982) Evolution in steppe with few, large, hooved mammals. *American Naturalist*, 119: 757-773.

Manner DJ (1985) Starch. In: Dey PM & Dixon RA (eds) *Biochemistry of storage carbohydrates in green plants*. Academic Press, London, pp 149-203.

Marbà N & Duarte CM (1998) Rhizome elongation and seagrass clonal growth. *Marine Ecology Progress Series*, 174: 269-280.

Marion B, Bonis A & Bouzillé JB (in prep. a) Plant diversity patterns in grazed and flooded grasslands: heterogeneity does not drive functional pattern as much as specific one.

Marion B, Bonis A & Bouzillé JB (in prep. b) Plant functional structure along grazing gradient: a community perspective taking into account intra-specific variability in traits.

Marion B, Bonis A & Bouzillé JB (submitted) How much does grazing-induced heterogeneity impact plant diversity and richness in a wet grassland?

Marshall C (1990) Source-sink relations of interconnected ramets. In: van Groenendael J & de Kroon H (eds) *Clonal growth in plants: regulation and function*. SPB Academic Publishing, The Hague, pp 23-41.

Maschinski J, Whitham TG (1989) The continuum of plant responses to herbivory: the influence of plant association, nutrient availability, and timing. *American Naturalist*, 134: 1-19.

McIntyre S, Lavorel S, Landsberg J & Forbes TDA (1999) Disturbance response in vegetation – toward a global perspective on functional traits. *Journal of Vegetation Science*, 10: 621-630.

McIntyre S, Lavorel S & Tremont RM (1995) Plant life-history attributes: their relationship to disturbance response in herbaceous vegetation. *Journal of Ecology*, 83: 31-44.

McLellan AJ, Prati D, Kaltz O & Schmid B (1997) Structure and analysis of phenotypic and genetic variation in clonal plants. In: de Kroon H & van Groenendael J (eds) *The ecology and evolution of clonal plants*. Backhuys Publishers, Leiden, pp 185-210.

McNaughton SJ (1979) Grazing as an optimization process: grass-ungulate relationships in the Serengeti. *American Naturalist*, 113: 691-703.

McNaughton SJ (1983) Compensatory plant growth as a response to herbivory. *Oikos*, 40: 329-336.

McNaughton SJ & Chapin III FS (1985) Effects of phosphorus nutrition and defoliation on C_4 graminoids from the Serengeti plains. *Ecology*, 66: 1617-1629.

Ménard C, Ducan P, Fleurance G, George JY & Lila M (2002) Comparative foraging and nutrition in horses and cattle in Europe wetlands. *Journal of Applied Ecology*, 38: 120-133.

Mesléard F, Lepart J, Grillas P & Mauchamp A (1999) Effects of seasonal flooding and grazing on the vegetation of former ricefields in the Rhône delta (Southern France). *Plant Ecology*, 145: 101-114.

Meuriot F, Avice JC, Simon JC, Lainé P, Decau ML & Ourry A (2004) Influence of initial organic N reserves and residual leaf area on growth, N uptake, N partitioning and N storage in alfalfa (*Medicago sativa*) during post-cutting regrowth. *Annals of Botany*, 94: 311-321.

Meyer AH & Schmid B (1999) Experimental demography of rhizome populations of establishing clones of *Solidago altissima*. *Journal of Ecology*, 87: 42-54.

Milchunas DG & Noy-Meir I (2002) Grazing refuges, external avoidance of herbivory and plant diversity. *Oikos*, 99: 113-130.

Milchunas DG, Sala OE & Lauenroth WK (1988) A generalized model of the effects of grazing by large herbivores on grassland community structure. *American Naturalist*, 132: 87-106.

Millard P (1988) The accumulation and storage of nitrogen by herbaceous plants. *Plant, Cell & Environment*, 11: 1-8.

Moen J, Ingvarsson PK & Walton DWH (1999) Estimates of structural complexity in clonal plant morphology: comparison of grazed and ungrazed *Acaena magellanica* rhizomes. *Canadian Journal of Botany*, 77: 869-876.

Mogie M & Hutchings MJ (1990) Phylogeny, ontogeny and clonal growth in vascular plants. In: van Groenendael J & de Kroon H (eds) *Clonal growth in plants: regulation and function*. SPB Academic Publishing, The Hague, pp 3-22.

Monro K, Poore AGB & Brooks R (2007) Multivariate selection shapes environment-dependent variation in the clonal morphology of a red seaweed. *Evolutionary Ecology*, 21: 765-782.

Monson RK, Rosenstiel TN, Forbis TA, Lipson DA & Jaeger CH III (2006) Nitrogen and carbon storage in alpine plants. *Integrative and Comparative Biology*, 46: 35-48.

Mony C, Garbey M, Smaoui M & Benot ML (submitted) Optimal trait profiles for clonal growth in plant species: a modeling approach.

Morvan-Bertrand A, Boucaud J, Le Saos J & Prud'homme MP (2001) Roles of fructans from leaf sheaths and from the elongating leaf bases in the

Morvan-Bertrand A, Boucaud J & Prud'homme MP (1999a) Influence of initial levels of carbohydrates, fructans, nitrogen, and soluble proteins on regrowth of *Lolium perenne* L. cv. Bravo following defoliation. *Journal of Experimental Botany*, 50: 1817-1825.

Morvan-Bertrand A, Pavis N, Boucaud J & Prud'homme MP (1999b) Partitioning of reserve and newly assimilated carbon in roots and leaf tissues of *Lolium perenne* during regrowth after defoliation : assessment by ^{13}C steady-state labelling and carbohydrate analysis. *Plant, Cell and Environment*, 22: 1097-1108.

Moussie AM, Apol MEF, Heil GW & van Diggelen R (2008) Creation and preservation of vegetation patterns by grazing. *Ecological Modelling*, 218: 60-72.

Moussie AM, Vos P, Verhagen HMC & Bakker JP (2005) Endozoochory by free-ranging, large herbivores: ecological correlates and perspectives for restoration. *Basic and Applied Ecology*, 6: 547-558.

Newton PCD & Hay MJM (1996) Clonal growth of white clover: factors influencing the viability of axillary buds and the outgrowth of a viable bud to form a branch. *Annals of Botany*, 78: 111-115.

Noy-Meir I & Briske DD (2002) Response of wild wheat populations to grazing in Mediterranean grasslands: the relative influence of defoliation, competition, mulch and genotype. *Journal of Applied Ecology*, 39: 259-278.

Noy-Meir I, Gutman M & Kaplan Y (1989) Responses of Mediterranean grassland plants to grazing and protection. *Journal of Ecology*, 77: 290-310.

Nzunda EF, Griffiths ME & Lawes MJ (2008) Sprouting by remobilization of above-ground resources ensures persistence after disturbance of coastal dune forest trees. *Functional Ecology*, 22: 577-582.

Oborny B & Cain ML (1997) Models of spatial spread and foraging in clonal plants. In: de Kroon H & van Groenendael J (eds) *The ecology and evolution of clonal plants*. Backhuys Publishers, Leiden, pp. 155-183.

Oborny B, Czárán T & Kun A (2001) Exploration and exploitation of resources patches by clonal growth: a spatial model on the effect of transport between modules. *Ecological Modelling*, 141: 151-169.

Oborny B & Kun A (2002) Fragmentation of clones: how does it influence dispersal and competitive ability? *Evolutionary Ecology*, 15: 319-346.

Oborny B, Kun A, Czárán T & Bokros S (2000) The effect of clonal integration on plant competition for mosaic habitat space. *Ecology*, 81: 3291-3304.

Oesterheld M & McNaughton SJ (1991) Interactive effect of flooding and grazing on the growth of Serengeti grasses. *Oecologia*, 88: 153-156.

Oksanen J, Kindt R, Legendre P, O'Hara B, Simpson GL, Solymos P, Stevens MHH & Wagner H (2008) Vegan: Community Ecology Package. R package version 1.15-1. http://cran.r-project.org/, http://vegan.r-forge.r-project.org.

Olff H & Ritchie ME (1998) Effects of herbivores on grassland plant diversity. *Trends in Ecology and Evolution*, 13: 261-265.

Olofsson J, de Mazancourt C & Crawley MJ (2008) Spatial heterogeneity and plant species richness at different spatial scales under rabbit grazing. *Oecologia*, 156: 825-834.

Olson BE & Richards JH (1988) Tussock regrowth after grazing: intercalary meristems and axillary bud activity of tillers of *Agropyron desertorum*. *Oikos*, 51: 374-382.

Oom SP, Sibbald AM, Hester AJ, Miller DR & Legg CJ (2008) Impacts of sheep grazing a complex vegetation mosaic: relating behavior to vegetation change. *Agriculture, Ecosystems and Environment*, 124: 219-228.

Ourry A, Boucaud J & Salette J (1988) Nitrogen mobilization from stubble and roots during re-growth of defoliated perennial ryegrass. *Journal of Experimental Botany*, 39: 803-809.

Owen D (1980) How plants may benefit from the animals that eat them. *Oikos*, 35: 230-235.

Oyarzabal M & Oesterheld M (2009) Phosphorus reserves increase grass regrowth after defoliation. *Oecologia*, 159: 717-724.

Paige KN & Whitham TG (1987) Overcompensation in response to mammalian herbivory: the advantage of being eaten. *American Naturalist*, 129: 407-416.

Pakeman RJ (2004) Consistency of plant species and trait responses to grazing along a productivity gradient: a multi-site analysis. *Journal of Ecology*, 92: 893-905.

Pakeman RJ, Lepš J, Kleyer M, Lavorel S, Garnier E & the VISTA consortium (2009) Relative climatic, edaphic and management controls of plant functional trait signatures. *Journal of Vegetation Science*, 20: 148–159.

Pakeman RJ, Pywell RF & Wells TCE (2002) Species spread and persistence: implications for experimental design and habitat re-creation. *Applied Vegetation Science*, 5: 75-86.

Pavis N, Chatterton NJ, Harrison PA, Baumgartner S, Praznik W, Boucaud J & Prud'homme MP (2001) Structure of fructans in roots and leaf tissues of *Lolium perenne*. *New Phytologist*, 150: 83-95.

Pennings S & Callaway R (2000) The advantages of clonal integration under different ecological conditions: a community-wide test. *Ecology*, 81: 709-716.

Piqueras J (1999) Herbivory and ramet performance in the clonal herb *Trientalis europara* L. *Journal of Ecology*, 87: 450-460.

Piqueras J, Klimeš L & Redbo-Torstensson P (1999) Modelling the morphological response to nutrient availability in the clonal plant Trientalis europaea L. Plant Ecology, 141: 117-127.

Piquot Y, Petit D, Valero M, Cuguen J, de Laguerie P & Vernet P (1998) Variation in sexual and asexual reproduction among young and old populations of the perennial macrophyte *Sparganium erectum*. *Oikos*, 82: 139-148.

Pitelka LF & Ashmun JW (1985) Physiology and integration of ramets in clonal plants. In: Jackson JBC, Buss LW & Cook RE (eds) *Population biology and evolution of clonal organisms*. Yale University Press, New Haven, pp 399-435.

Pollock CJ & Cairns AJ (1991) Fructan metabolism in grasses and cereals. *Annual Review of Plant Physiology and Plant Molecular Biology*, 42: 77-101.

Pontis HG & del Campillo E (1985) Fructans. In: Dey PM & Dixon RA (eds) *Biochemistry of storage carbohydrates in green plants*. Academic Press, London, pp 205-227.

Posse G, Anchorena J & Collantes MB (2000) Spatial micro-patterns in the steppe of Tierra del Fuego induced by sheep grazing. *Journal of Vegetation Science*, 11: 43-50.

Prati D & Schmid B (2000) Genetic differentiation of life-history traits within population of the clonal plant *Ranunculus reptans*. *Oikos*, 90: 442-456.

Price EAC, Gamble R, Williams GG & Marshall C (2002) Seasonal patterns of partitioning and remobilization of ^{14}C in the invasive rhizomatous perennial Japanese knotweed (*Fallopia japonica* (Houtt.) Ronse Decraene). *Evolutionary Ecology*, 15: 347-362.

Price EAC & Hutchings MJ (1992a) The causes and developmental effects of integration and independence between different parts of Glechoma hederacea clones.*Oikos*, 63: 376-386.

Price EAC & Hutchings MJ (1992b) Studies of growth in the clonal herb *Glechoma hederacea*. II. The effect of selective defoliation. *Journal of Ecology*, 80: 39-47.

Price EAC, Hutchings MJ (1996) The effects of competition on growth and form in *Glechoma hederacea*. *Oikos*, 75: 279-290.

Price EAC & Marshall C (1999) Clonal plants and environmental heterogeneity. An introduction to the proceedings. *Plant Ecology*, 141: 3-7.

Price EAC, Marshall C & Hutchings MJ (1992) Studies of growth in the clonal herb Glechoma hederacea. I. Patterns of physiological integration. *Journal of Ecology*, 80: 25-38.

Puijalon S, Bornette G & Sagnes P (2005) Adaptations to increasing hydraulic stress: morphology, hydrodynamics and fitness of two higher aquatic plant species. *Journal of Experimental Botany*, 56: 777-786.

Puijalon S, Bouma TJ, van Groenendael J & Bornette G (2008) Clonal plasticity of aquatic plant species submitted to mechanical stress: escape versus resistance strategy. *Annals of Botany*, 102: 989-996.

R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Reich PB, Ellsworth DS, Walters MB Vose JM, Gresham C, Volin JC & Bowman WD (1999) Generality of leaf trait relationships: a test across six biomes. *Ecology*, 80: 1955-1969.

Richards JH (1993) Physiology of plants recovering from defoliation. In: Baker MJ (ed) *Proceedings of the XVII International Grassland Congress*. SIR Publishing, Wellington, pp 85-94.

Richards JH, Mueller RJ & Mott JJ (1988) Tillering in tussock grasses in relation to defoliation and apical bud removal. *Annals of Botany*, 62: 173-179.

Rietkerk M, Ketner P, Burger J, Hoorens B & Olff H (2000) Multiscale soil and vegetation patchiness along a gradient of herbivore impact in a semi-arid grazing system in West-Africa. *Plant Ecology*, 148: 207-224.

Roiloa SR & Retuerto R (2006) Small-scale heterogeneity in soil quality influences photosynthetic efficiency and habitat selection in a clonal plant. *Annals of Botany*, 98: 1043-1052.

Rossignol N (2006) *Hétérogénéité de la végétation et du pâturage : conséquences fonctionnelles en prairies naturelles.* Thèse de 3ème cycle, Université de Rennes 1.

Rossignol N, Bonis A & Bouzillé JB (2006) Relationships between grazing pattern and vegetation structure on the spatial variations of net N mineralisation in a wet grassland. *Applied Soil Ecology*, 31: 62-72.

Rotundo JL & Aguiar MR (2008) Herbivory resistance traits in populations of *Poa ligularis* subjected to historically different sheep grazing pressure in Patagonia. *Plant Ecology*, 194: 121-133.

Sachs T & Novoplansky A (1997) What does aclonal organization suggest concerning clonal plants? In: de Kroon H & van Groenendael J (eds) *The ecology and evolution of clonal plants*. Backhuys Publishers, Leiden, pp. 55-77.

Sala OE, Oesterheld M, León RJC & Soriano A (1986) Grazing effects upon plant community structure in subhumid grasslands of Argentina. *Vegetatio*, 67: 27-32.

Sammul M, Kull K, Niitla T & Möls T (2004) A comparison of plant communities on the basis of their clonal growth patterns. *Evolutionary Ecology*, 18: 443-467.

Sammul M, Kull K & Tamm A (2003) Clonal growth in a species-rich grassland: results of a 20-year fertilization experiment. *Folia Geobotanica*, 38: 1-20.

Santamaria L (2002) Why are most aquatic plants widely distributed? Dispersal, clonal growth and small-scale heterogeneity in a stressful environment. *Acta Oecologica*, 23: 137-154.

Schmid B (1990) Some ecological and evolutionary consequences of modular organization and clonal growth in plants. *Evolutionary Trends in Plants*, 4: 25-34.

Schmid B & Bazzaz FA (1990) Plasticity in plant size and architecture in rhizome-derived *vs*. seed derived *Solidago* and *Aster*. *Ecology*, 71: 523-535.

Schnyder H & de Visser R (1999) Fluxes of reserve-derived and currently assimilated carbon and nitrogen in perennial ryegrass recovering from defoliation. The regrowing tiller and its component functionally distinct zones. *Plant Physiology*, 119: 1423-1435.

Schwaegerle KE, McIntyre H & Swingley C (2000) Quantitative genetics and the persistence of environmental effects in clonally propagated organisms. *Evolution*, 54: 452-461.

Schwinning S & Parsons AJ (1999) The stability of grazing system revisited: spatial models and the role of heterogeneity. *Functional Ecology*, 13: 737-747.

Scofield GN, Ruuska SA, Aoki N, Lewis DC, Tabe LM & Jenkins CLD (2009) Starch storage in the stems of wheat plants: localization and temporal changes. *Annals of Botany*, 103: 859-868.

Sintes T, Marba N, Duarte CM & Kendrick GA (2005) Nonlinear processes in seagrass colonisation explained by simple clonal growth rules. *Oikos*, 108: 165-175.

Sintes T, Moragues E, Traveset A & Rita J (2007) Clonal growth dynamics of the invasive *Carpobrotus affine acinaciformis* in Mediterranean coastal systems: a non-linear model. *Ecological Modelling*, 206, 110-118.

Slade AJ & Hutchings MJ (1987a) The effect of nutrient availability on foraging in the clonal herb *Glechoma hederacea*. *Journal of Ecology*, 75: 95-112.

Slade AJ & Hutchings MJ (1987b) The effects of light intensity on foraging in the clonal herb *Glechoma hederacea*. *Journal of Ecology*, 75: 639-650.

Smaoui M, Garbey M & Mony C (2008) Volunteer computing for ecology: the Virtual Prairie BOINC project. Paper presented in IEEE Asia-Pacific Services Computing Conference (IEEE APSCC 2008), Taiwan, pp 427-434.

Smith AP & Palmer JO (1976) Vegetative reproduction and close packing in a successional plant species. *Nature*, 261: 232-233.

Smith SE (1998) Variation in response to defoliation between populations of *Bouteloua curtipendula* var. *caespitosa* (Poaceae) with different livestock grazing histories. *American Journal of Botany*, 85: 1266-1272.

Sokal RR & Oden NJ (1978) Spatial autocorrelation in biology 1. Methodology. *Biological Journal of the Linnean Society*, 10: 199-228.

Song M & Dong M (2002) Clonal plants and plant species diversity in wetland ecosystems in China. *Journal of Vegetation Science*, 13: 237-244.

Sosnová M, van Diggelen R & Klimešová (2009) Distribution of clonal growth forms in wetlands. Aquatic Botany, 92: 33-39.

Soukupova L (1994) Allocation plasticity and modular structure in clonal graminoids in response to waterlogging. *Folia Geobotanica and Phytotaxonomica*, 29: 227-236.Steen E & Larsson K (1986) Carbohydrates in roots and rhizomes of perennial grasses. *New Phytologist*, 104: 339-346.

Stearns SC (1976) Life-history tactics: a review of the ideas. *The Quarterly Review of Biology*, 51: 3-47.

Sternberg M, Gutman M, Perevolotsky A, Ungar ED & Kigel J (2000) Vegetation response to grazing management in a Mediterranean herbaceous community: a functional group approach. *Journal of Applied Ecology*, 37: 224-237.

Stöcklin J & Winkler E (2004) Optimum reproduction and dispersal strategies of a clonal plant in a metapopulation: a simulation study with *Hieracium pilosella*. *Evolutionary Ecology*, 18: 563-584.

Stohlgren TJ, Schell LD & Vanden Heuvel BV (1998) How grazing and soil quality affect native and exotic plant diversity in Rocky Mountain grasslands. *Ecological Applications*, 9: 45-64.

Stoll P, Egli P & Schmid B (1998) Plant foraging and rhizome growth patterns of *Solidago altissima* in response to mowing and fertilizer application. *Journal of Ecology*, 86: 341-354.

Stowe KA, Marquis RJ, Hochwender CG & Simms EL (2000) The evolutionary ecology of tolerance to consumer damage. *Annual Review of Ecology and Systematics*, 31: 565-595.

Strauss SY & Agrawal AA (1999) The ecology and evolution of plant tolerance to herbivory. *Trends in Ecology and Evolution*, 14: 179-185.

Stuefer JF (1996) Potential and limitations of current concepts regarding the response of clonal plants to environmental heterogeneity. *Vegetatio*, 127: 55-70.

Stuefer JF (1998) Two types of division of labour in clonal plants: benefits, costs and constraints. *Perspectives in Plant Ecology, Evolution and Systematics*, 1: 47-60.

Stuefer JF, Gómez S & van Mölken T (2004) Clonal integration beyond resource sharing: implications for defence signalling and disease transmission in clonal plant networks. *Evolutionary Ecology*, 18: 647-667.

Stuefer JF & Huber H (1998) Differential effects of light quantity and spectral light quality on growth, morphology and development of two stoloniferous *Potentilla* species. *Oecologia*, 117: 1-8.

Stuefer JF & Huber H (1999) The role of stolon internodes for ramet survival after clone fragmentation in *Potentilla anserina*. *Ecology Letters*, 2: 135-139.

Sultan SE & Spencer HG (2002) Metapopulation structure favors plasticity over local adaptation. *American Naturalist*, 160: 271-283.

Sutherland WJ & Stillman RA (1988) The foraging tactics of plants. Oikos, 52: 239-244.

Suzuki JI & Hutchings MJ (1997) Interactions between shoots in clonal plants and the effect of stored resources on the structure of shoot populations. In: de Kroon H & van Groenendael J (eds) *The ecology and evolution of clonal plants*. Backhuys Publishers, Leiden, pp 311-329.

Suzuki JI & Stuefer J (1999) On the ecological and evolutionary significance of storage in clonal plants. *Plant Species Biology*, 14: 11-17.

Tamm A, Kull K & Sammul M (2002) Classifying clonal growth forms based on vegetative mobility and ramet longevity: a whole community analysis. *Evolutionary Ecology*, 15: 383-401.

ter Braak CJF & Šmilauer P (1998) *CANOCO Reference Manual and User's Guide to Canoco for Windows: Software for Canonical Community Ordination (version 4).* Micro Computer Power, New York, Ithaca

Thioulouse J, Chessel D, Dodélec S & Olivier JM (1997) ADE-4: a multivariate analysis and graphical display software. *Statistical Computing*, 7: 75-83.

Thomas H & James AR (1999) Partitioning of sugars in *Lolium perenne* (perennial ryegrass) during drought and on rewatering. *New Phytologist* 142: 292-305.

Thornton B, Millard P, Duff EI & Buckland ST (1993) The relative contribution of remobilization and root uptake in supplying nitrogen after defoliation for regrowth of laminae in four grass species. *New Phytologist*, 124: 689-694.

Tiffin P (2000) Mechanisms of tolerance to herbivore damage: what do we know? *Evolutionary Ecology*, 14: 523-536.

Tilman D (2001) Functional diversity. In: Levin SA (ed) *Encyclopedia of biodiversity*. Academic Press, San Diego, pp 109-120.

Tomlinson KW, Dominy JG, Hearne JW & O'Connor TG (2007) A functional-structural model for growth of clonal bunchgrasses. *Ecological Modelling*, 202: 243-264.

Tuomi J, Nilsson P & Åström M (1994) Plant compensatory responses: bud dormancy as an adaptation to herbivory. *Ecology*, 75: 1429-1436.

Tuomi J & Vuorisalo T (1989) What are the units of selection in modular organisms? *Oikos*, 54: 227-233.

van der Meidjen E, de Boer NJ & van der Veen-van Wijk (2000) Pattern of storage and regrowth in ragwort. *Evolutionary Ecology*, 14: 439-455.

van der Meijden E, Wijn M & Verkaar H (1988) Defence and regrowth, alternative plant strategies in the struggle against herbivores. *Oikos*, 51: 355-363.

van Eck WHJM, van de Steeg HM, Blom CWPM & de Kroon H (2004) Is tolerance to summer flooding correlated with distribution patterns in river floodplains? A comparative study of 20 terrestrial grassland species. *Oikos*, 107: 393-405.

van Groenendael J & de Kroon H (1990) *Clonal growth in plants: regulation and function.* SBP Academic Publishing, The Hague.

van Groenendael JM, Klimeš L, Klimešovà J & Hendricks R (1996) Comparative ecology of clonal plants. *Philosophical Transactions of the Royal Society London*, 351: 1331-1339.

van Kleunen M & Fischer M (2001) Adaptive evolution of plastic foraging responses in a clonal plant. *Ecology*, 82: 3309-3319.

Veen GF, Blair JM, Smith MD & Collins SL (2008) Influence of grazing and fire frequency on small-scale plant community structure and resource variability in native tallgrass prairie. *Oikos*, 117: 859-866.

Verdaguer D & Ojeda F (2002) Root starch storage and allocation patterns in seeder and resprouter seedlings of two Cape *Erica* (Ericaceae) species. *American Journal of Botany*, 89: 1189-1196.

Vesk PA & Westoby M (2001) Predicting plant species' responses to grazing. *Journal of Applied Ecology*, 38: 897-909.

Vuorisalo T & Tuomi J (1986) Unitarity and modular organisms: criteria for ecological division. *Oikos*, 47: 382-385.

WallisDeVries MF, Laca EA & Demment MW (1999) The importance of scale of patchiness for selectivity in grazing herbivores. *Oecologia*, 121: 355-363.

Wang Z, Li L, Han X & Dong M (2004) Do rhizome severing and shoot defoliation affect clonal growth of *Leymus chinensis* at ramet population level ? *Acta Oecologica*, 26: 255-260. Wang Z, Li L, Han X & Dong M (2004) Do rhizome severing and shoot defoliation affect clonal growth of *Leymus chinensis* at ramet population level ? *Acta Oecologica*, 26: 255-260.

Wardle DA, Barker GM, Yeates GW, Bonner KI & Ghani A (2001) Introduced browsing mammals in New Zealand natural forests: aboveground and belowground consequences. *Ecological Monographs*, 71: 587-614.

Watson MA & Casper Casper BB (1984) Morphogenetic constraints on patterns of carbon distribution in plants. Annual Review of Ecology and Systematics, 15: 233-258.

Weber GE, Jeltsch F, van Rooyen N & Milton SJ (1998) Simulated long-term vegetation response to grazing heterogeneity in semi-arid rangelands. *Journal of Applied Ecology*, 35: 687-699.

Weiher E, van der Werf A, Thompson K, Roderick M, Garnier E & Eriksson O (1999) Challenging Theophrastus: a common core list of plant traits for functional ecology. *Journal of Vegetation Science*, 10: 609-620.

Wennström A & Ericson L (1992) Environmental heterogeneity and disease transmission within clones of *Lactuca sibirica*. *Journal of Ecology*, 80: 71–77.

Westoby M (1998) A leaf-height-seed (LHS) plant ecology strategy scheme. *Plant and Soil*, 199: 213-227.

Wijesinghe DK & Hutchings MJ (1997) The effects of spatial scale of environmental heterogeneity on the growth of a clonal plant: an experimental study with *Glechoma hederacea*. *Journal of Ecology*, 85: 17-28.

Wijesinghe DK & Hutchings MJ (1999) The effects of environmental heterogeneity on the performance of *Glechoma hederacea*: the interactions between patch contrast and patch scale. *Journal of Ecology*, 87: 860-872.

Wikberg S (1995) Fitness in clonal plants. *Oikos*, 72: 293-297.

Wildová R (2004) Below-ground spatial pattern of rhizomes in a grassland community and its relevance to above-ground spatial pattern. *Plant Ecology*, 174: 319-336

Wildová R, Gough L, Herben T, Hershock C & Goldberg DE (2007a) Architectural and growth traits differ in effects on performance of clonal plants: an analysis using a field-parameterized simulation model. *Oikos*, 116: 836-852.

Wildová R, Wild J & Herben T (2007b) Fine-scale dynamics of rhizomes in a grassland community. *Ecography*, 30: 264-276.

Winkler E & Fischer M (1999) Two fitness measures for clonal plants and the importance of spatial aspects. *Plant Ecology*, 141: 191-199.

Winkler E & Fischer M (2002) The role of vegetative spread and seed dispersal for optimal life histories of clonal plants: a simulation study. *Evolutionary Ecology*, 15: 281-301.

Wolfer SR, van Nes EH & Straile D (2006) Modelling the clonal growth of the rhizomatous macrophyte *Potamogeton perfoliatus*. *Ecological Modelling*, 192: 67-82.

Zhou Z, Sun OJ, Luo Z, Jin H, Chen Q & Han X (2008) Variation in small-scale spatial heterogeneity of soil properties and vegetation with different land use in semiarid grassland ecosystem. *Plant Soil*, 310: 103-112.

Zobel M. (2008) On the forces that govern clonality versus sexuality in plant communities. *Evolutionary Ecology*, 22: 487-492.

ANNEXE – CONSEQUENCE OF RAMET DEFOLIATION ON PLANT CLONAL PROPAGATION AND BIOMASS ALLOCATION: EXEMPLE OF FIVE RHIZOMATOUS GRASSLAND SPECIES.

Majid Mohammad-Esmaeili, Anne Bonis, Jan-Bernard Bouzillé, Cendrine Mony, & Marie-Lise Benot

UMR CNRS 6553, ECOBIO, Université de Rennes 1, Campus de Beaulieu, 35042 Rennes Cedex, France

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